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POTASSIUM DEFICIENCY IN SUGAR CANE

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CONSTANCE ENDICOTT HARTT

(WITH FOURTEEN FIGURES)

Potassium has been included in lists of essential elements since 1804, when the necessity of potassium salts for terrestrial plants was established by DE SAUSSURE (6). Since then attention has been focused upon the rôle of potassium, but its exact function has not yet been definitely determined. A good general review of the literature dealing with potassium is given by RUSSELL (29). The work reported here is an attempt: (1) to obtain gradations in growth correlated with gradations in the potassium supplied in the nutrient solutions; and (2) to secure, if possible, correlations with physiological processes, especially enzyme activity.

The literature contains many references to experiments with prepared enzymes, for example, Taka-diastase and Merck's diastase of malt. Such material has not been prepared from plants grown in a potassium-free nutrient solution, and it is generally made from seedlings which are too young to show potassium starvation phenomena. To determine the effect of potassium upon enzymes it is really necessary to use material prepared from plants starved for potassium, compared with controls. This is the method of DOBY and HIBBARD (7), and has been used in the present investigation.

Methods

1. Cultural

Cuttings of Cheribon (Louisiana Purple) sugar cane, obtained from Dr. E. W. Brandes of the United States Department of Agriculture, were used in this investigation. Similar experiments with wheat, buckwheat, and soy bean will be reported in a later paper.

Twelve healthy cuttings of sugar cane with roots already starting were covered with sterilized quartz sand in a flat on October 25, 1927. They were watered well with distilled water and placed in a warm greenhouse. The first sprout appeared in three days and was quickly followed by others. They were allowed to become well rooted before being moved. On November 22 the plants were cut from the seed pieces with a sterilized knife and planted in 2-gallon glazed earthenware crocks, one plant in each crock. About 2 inches of seed piece remained on each plant. The plants were watered with distilled water for three days and then, since no mortality occurred due to the transplanting, watering with the solutions was begun. Artificial light was used during the winter to supplement the daylight (two 200 watt bulbs with reflectors over approximately the upper third of the bulbs).

The quartz sand was sterilized in 20 per cent hydrochloric acid followed by thorough washing with distilled water. The solutions were made up on the basis of Shive's (30) best, which is a 3-salt solution with an osmotic concentration of 1.75 atmospheres. Plants were grown with four different amounts of potassium, equivalent amounts of sodium being added to replace the potassium and maintain the solutions at equal osmotic pressure. Iron was added as ferric phosphate according to the directions of Livingston (18). The following nutrient solutions were prepared from volume molecular stock solutions, each made up to a liter:

Solution I (Shive's best) control: 18 cc. KH_2PO_4+5.2 cc. $Ca(NO_3)_2.4\ H_2O$ +15 cc. $MgSO_4.7\ H_2O$.

Solution II: 18 cc. $NaH_2PO_4.H_2O$+5.2 cc. $Ca(NO_3)_2.4\ H_2O$+15 cc. $MgSO_4.7\ H_2O$.

Solution III: 17.9 cc. $NaH_2PO_4.H_2O$+0.1 cc. KH_2PO_4+5.2 cc. $Ca(NO_3)_2.4\ H_2O$+15 cc. $MgSO_4.7\ H_2O$.

Solution IV: 17 cc. $NaH_2PO_4.H_2O$+1 cc. KH_2PO_4+5.2 cc. $Ca(NO_3)_2.4\ H_2O$ +15 cc. $MgSO_4.7\ H_2O$.

Three plants were grown with solution I and four with each of the others. Throughout this report numerals I–IV will designate the plants grown in these respective solutions. The plants were watered with these solutions as needed. Drainage was maintained by bent Pyrex tubes inserted through the opening in the bottom of each crock, which operated as siphons and could be closed with pinch clamps.

II. Collection and preparation

One plant of each group was collected early in the afternoon of April 14 and taken immediately to the laboratory, where measurements of size, weight, etc., were made. The tops were removed, ground rapidly in a Russwin mill, and the pulp thoroughly mixed. Duplicate portions were immediately weighed and set aside in watch glasses with ground glass edges, held together with clips, for moisture determinations. Duplicate portions for sugar determinations were weighed in Erlenmeyer flasks containing a little calcium carbonate and boiled in 95 per cent alcohol. The rest of the material was placed in evaporating dishes, covered with several layers of cheesecloth and evaporated to dryness with an electric fan. It was then stored in desiccators. The roots were dried without being ground.

The second collection was made early in the afternoon of June 2. The same procedure was followed except that the leaf blades were separated from the sheaths, the latter being included with the stems.

III. Quantitative determinations of enzyme activity

The diastase activity was determined quantitatively by the rate of disappearance of starch as shown by the color with IKI.

Quantitative determinations of the activity of invertase were made by measuring the increase, during 24 hours, in the ability to reduce Fehling's solution. The reducing action was determined by the method of Munson and Walker (**23**). The Bertrand method (3), titration with potassium permanganate, was used for estimating the amount of copper oxide formed in the Fehling's solution. The relative activity of peptase was estimated by comparing the amounts of color liberated from carmine fibrin by equal amounts of material in a given time and under the same conditions. The carmine fibrin

was prepared according to the directions given by HAWK (11), and is a substrate suggested by GRÜTZNER (8).

The formation of tryptophane from Witte peptone as discussed by VINES (32) was used to estimate the activity of ereptase. The tryptophane was dissolved in bromine water and treated with amyl alcohol. The activity of catalase was determined by the liberation of oxygen from hydrogen peroxide by the method of APPLEMAN (1).

IV. POTASSIUM DETERMINATION

Potassium was determined by the cobaltinitrite method, the precipitate being measured by titration with potassium permanganate. The micromethod of KRAMER and TISDALL (17) as modified by KERR (15) was employed.

V. SUGARS

Reducing substances were determined by the MUNSON and WALKER method (23). The amount of copper oxide formed in the Fehling's solution was measured by the BERTRAND method (3). The citric acid inversion method described by DAVIS and DAISH (5) was used for determining the total sugars.

VI. MICROCHEMICAL TESTS

The tests used for cellulose were methylene blue and chlorozinc iodide. Orcin and phoroglucin were employed to test for lignin. Calcium pectate was stained by means of methylene blue and ruthenium red. Tests for cutin were made with Sudan III.

Results

I. GROWTH

The growth rate of the plants in all the solutions remained equal until March, by the end of which month a decided gradation in size was apparent. The rate remained equal while the light was deficient, but when the days became longer and sunnier the plants receiving potassium began to grow more rapidly, while the potassium-deficient plants gradually ceased to grow.

The starvation symptoms were small size and dieback, the latter particularly of the lower leaves. Figs. 1 and 2 show their appearance on April 12. Measurements of single plants made on April 14 are

recorded in table I. The length of the tops was obtained by stretching them out and measuring to the tip of the longest leaf. Breadth of

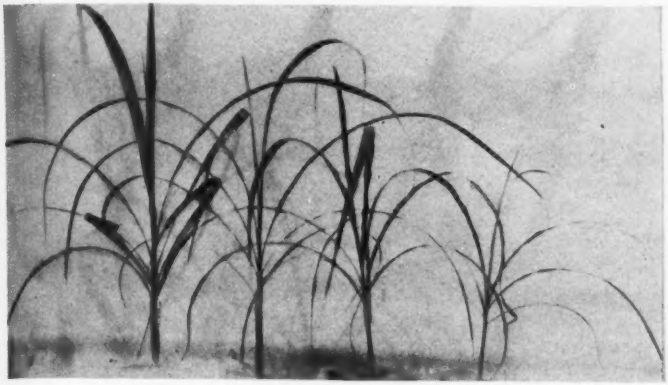


FIG. 1.—Appearance of sugar cane plants on April 12, 5.5 months old; left to right: I, IV, III, II.

FIG. 2.—Sugar cane plants on April 12; left to right: I, II

leaf is a measurement taken at the middle of the largest leaf. The differences in the weights of the roots are more apparent than real

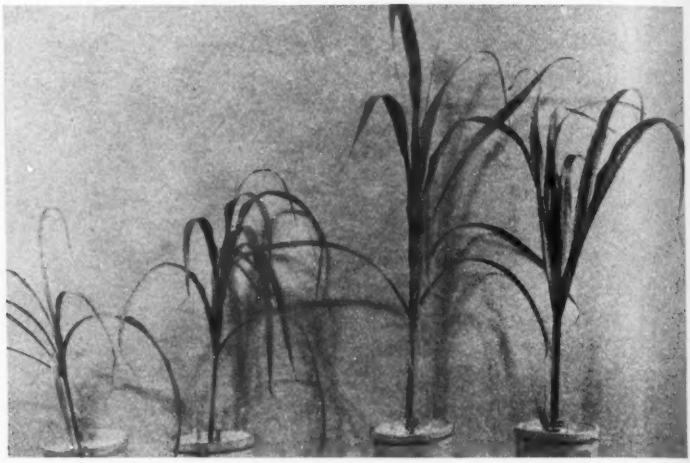


FIG. 3.—Sugar cane plants in June, 7 months old; left to right: II, III, IV, I

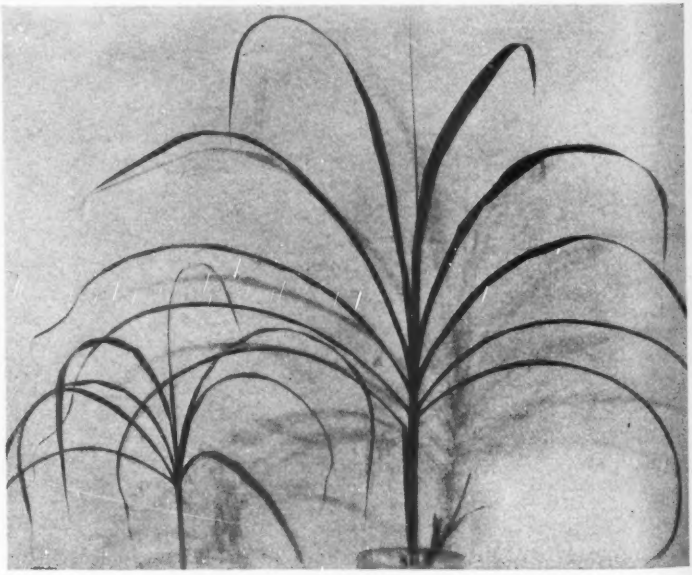


FIG. 4.—Sugar cane plants in June; left to right: II, IV

because some sand clung to them. This difficulty was obviated in later work by rinsing in 10 per cent sodium chloride.

At the time of collection a great difference in the roots was observed. The roots of plant I were glistening white and had many fine

TABLE I

MEASUREMENTS OF CANE PLANTS COLLECTED APRIL 14

PLANT	WEIGHT TOPS (GM.)	WEIGHT ROOTS (GM.)	LENGTH TOPS (IN.)	LENGTH ROOTS (IN.)	LEAF BREADTH (CM.)	STEM GIRTH (CM.)
I.....	110.5	48.0	67.0	20	3.6	4.9
II.....	31.5	12.0	55.0	25	2.1	3.0
III.....	51.0	14.9	59.5	35	2.7	3.4
IV.....	54.7	26.0	56.5	17	2.6	3.4

branches forming a dense system. They were tough and strong and lifted completely out of the crock without breaking. Contrasting with this were the roots of plant II, which were discolored, brownish, and were thin with the fibrous roots underdeveloped. These broke easily and it was very difficult to remove them from the crock. Notwithstanding the fact that the tips broke off, the longest roots of II, measured as a mass, were 25 inches in length while those of I were only 20 inches long. The roots of plant III were a little stronger than those of II but showed similar discoloration. Plant IV, however, had roots almost as strong and white as plant I. Although the roots of plant IV measured half as long as plant III, they weighed almost twice as much.

FIG. 5.—Roots of plant I as removed from crock on June 2, showing potbound condition.

The second collection was made on June 2, when the plants appeared as in figs. 3 and 4. When the roots were removed it was found that plant I was potbound (fig. 5). Table II shows their fresh weight and table III their size. The roots were washed in 10 per cent sodium chloride and then distilled water. The symptoms of potassium starvation noticed at the time of the first collection were small size and

dieback. To these was added another symptom by the time of the second collection, discoloration of the leaves. The upper leaves of plant II were decidedly yellower than the lower leaves. This yellow discoloration was confined to the tissue between the veins, which were green. The other plants were green without discoloration. The

TABLE II

WEIGHT OF CANE PLANTS COLLECTED JUNE 2, EXPRESSED IN GRAMS

PLANT	TOPS	BLADES	SHEATHS AND STEMS	ROOTS
I	187.0	76.0	111.0	118.5
II	64.0	40.0	24.0	43.0
III	105.5	63.0	42.5	102.0
IV	178.0	96.7	81.3	106.8

TABLE III

MEASUREMENTS OF CANE PLANTS COLLECTED JUNE 2

PLANT	LENGTH TOPS (IN.)	LENGTH ROOTS (IN.)	LEAF BREADTH (CM.)	STEM GIRTH (CM.)	NODAL GIRTH (CM.)
I	64.5	19.0	4.9	7.2	6.5
II	57.2	26.2	2.4	4.5	2.8
III	63.2	29.2	3.3	5.0	3.9
IV	65.2	21.5	4.0	6.1	5.0

incrustation of wax in the region of the nodes was much less in plant II than in the other plants.

The last collection was made July 12, at which time the leaves of plant II were conspicuously yellow while the others were green. The stems showed a gradation in size, as illustrated in fig. 6. This material was used for microchemical tests.

II. ENZYME RESULTS

A. DIASTASE

Tops collected April 14.—As this experiment was performed three times with similar results, an account of a typical set will be given. Lots of powder weighing 0.04 gm. from each plant were extracted in 4 cc. H_2O at room temperature for four hours. To these 1 per cent enzyme solutions were then added 5 cc. of 1 per cent starch solution plus 0.5 cc. toluol and enough sodium phosphate to make them pH 4.4. The 1 per cent solution of soluble starch was prepared according

to the directions given by WAKSMAN and DAVISON (33). The cultures were incubated at 42° C. for 24 hours, at which time the results

FIG. 6.—Stems of plants collected July 12, 8.5 months old; left to right: I, IV, III, II

shown in table IV were obtained. Since the tube containing material of plant II became red upon the addition of IKI, while that of plant I

turned blue-purple, it was concluded that the diastase activity of the potassium-deficient plant was greater than that of the control.

TABLE IV

DIASTASE ACTIVITY AS SHOWN BY COLOR WITH IKI

PLANT	TOPS, APRIL COLLECTION	BLADES, JUNE COLLECTION	STEMS AND SHEATHS, JUNE COLLECTION
I	Blue-purple	Brown-red	Dark purple
II	Red	Pink-brown	Yellow
III	Purple-red	Pink-brown	Yellow
IV	Blue-purple	Red	Red

Plant III was intermediate in activity, while plant IV closely resembled plant I.

Plants collected June 2.—One per cent solutions of enzymes prepared from blades and stems plus sheaths, extracted one-half hour and incubated 24 hours at 40° C., gave the results shown in table IV. The coloration produced by the addition of IKI showed that the potassium-deficient plants had greater diastase activity than the controls, both in the blades and in the stems plus sheaths. The roots of the plants collected June 2, tested in the same way, all gave a dark red color, indicating uniform diastase activity.

B. INVERTASE

0.1 gm. lots of powder plus 5 cc. H_2O plus 5 cc. 3 per cent sucrose plus 0.5 cc. toluol, incubated 24 hours at 41° C., gave the results presented in table V. The amount of invert sugar formed by the invertase of the tops collected in April was about the same in all the plants, showing that the invertase activity of the plants did not vary at that age. By the time of the June collection the activity of the

TABLE V

INVERT SUGAR FORMED BY INVERTASE

PLANT	TOPS, APRIL COLLECTION (MG.)	BLADES, JUNE COLLECTION (MG.)	STEMS AND SHEATHS, JUNE COLLECTION (MG.)
I	22.1	42.8	35.4
II	21.6	18.0	39.7
III	19.3	22.0	40.1
IV	23.2	20.6	31.7

blades of plant I had almost doubled, while that of the other plants remained about the same. The activity of the stems and sheaths had also increased by June, but more uniformly than the blades, since the plants receiving the intermediate amounts of potassium, were the extremes in activity.

c. Peptase

Preliminary tests showed pH 8 to be a favorable reaction for cane peptase. This reaction was therefore maintained in the experiment by means of sodium hydroxide with sodium phosphate as buffer.

Cane tops.—0.1 gm. lots of powder plus 5 cc. H_2O plus 0.1 gm. carmine fibrin plus 0.5 cc. toluol at pH 8, incubated at 40° C., began to develop a faint pink color in 3 days. The test was discontinued at

TABLE VI

Color released from carmine fibrin by peptase in 5 days

Plant	Blades	Stems and sheaths
I.	Deepest pink	Deepest pink
II.	Pink	Pink
III.	Deeper pink	Pink
IV.	Deepest pink	Deeper pink

the end of one week because the color in all the tubes deepened uniformly, indicating equal activity.

Cane blades and stems plus sheaths.—These, tested as indicated, began to show a faint pink color in 2 days. The color did not deepen uniformly, but gave in 5 days the gradation shown in table VI, proving that the plants supplied with potassium had greater peptase activity than the potassium-deficient plants.

Cane roots.—These, tested as indicated, liberated color much more rapidly than the tops. In seven hours I and IV were pink but II and III remained light yellow; therefore the peptase activity of cane roots is greater than that of the tops, and both the roots and the tops have greater activity when they have been supplied with potassium.

d. Ereptase

Preliminary tests showed pH 4.8 favorable for ereptase, and this reaction was therefore maintained in the experiments.

Cane tops.—0.02 gm. lots of powder plus 5 cc. H_2O plus 0.1 gm. Witte peptone plus 0.5 cc. toluol at pH 4.8 were incubated 3 days at 40° C. When tested with bromine water and amyl alcohol the four lots showed equal development of color, hence equal production of tryptophane and equal activity of ereptase.

Cane blades.—These, tested as indicated, all gave equally good tryptophane reactions, indicating equal activity of ereptase.

Cane stems plus sheaths.—These gave strong tryptophane reactions in plants II, III, and IV but only slight in I.

TABLE VII

OXYGEN LIBERATED BY CATALASE IN 0.1 GM. MATERIAL

PART	MINUTES	I (cc.)	II (cc.)	III (cc.)	IV (cc.)
Tops, April 14	1	2.27	0.66	0.82	0.27
	2	4.31	0.91	0.91	0.45
	5	6.81	1.18	1.18	0.75
	10	8.76	1.18	1.18	0.96
Blades, June 2	1	15.06	3.34	6.40	10.10
	2	21.38	4.15	8.21	12.90
	5	30.40	5.05	10.19	16.93
	10	39.24	5.68	11.82	21.02
Stems and sheaths, June 2	1	29.46	9.97	6.05	21.54
	2	42.45	13.17	8.54	28.93
	5	62.66	17.18	10.95	38.09
	10	77.70	20.56	12.99	49.31
Roots, April 14	1	2.36	2.45	2.27	1.73
	2	3.27	3.00	2.55	2.73
	5	4.82	3.64	2.91	4.36
	10	6.00	4.00	3.73	5.73

E. CATALASE

The amount of oxygen liberated from 10 cc. of 3 per cent hydrogen peroxide by 0.1 gm. material was measured in 1, 2, 5, and 10-minute intervals, in duplicate. The results were averaged and calculated to standard temperature and pressure and are given in table VII. This shows that the control plants had the greatest catalase activity, and that in general the gradation in activity was correlated with the gradation in the amount of potassium supplied. A notable exception was found in the stems and sheaths, in which

the catalase of plant II was decidedly more active than that of plant III.

III. Effect of equalizing potassium on invertase of cane blades

As shown in table V, the blades of plant I had an invertase activity about double that of the other plants. The question arose as to whether potassium is important for the formation of invertase in cane blades, or merely for its activity. To decide this point, potassium determinations in plants I and II were made, and then enough potassium phosphate was added to plant I to equalize the potassium in both materials. Potassium determinations were made in duplicate, and it was found that the dry weight percentage of potassium of plant I was 3.616 while that of plant II was only 0.497. This necessitated the addition of 1.04 cc. M/10 KH_2PO_4 to 0.1 gm. blade material of plant II and an equivalent amount of sodium phosphate to plant I (to regulate the pH and the osmotic concentration). Blanks were run on both the phosphates. The results, which are given in table VIII, show that even after equalization of the potassium, the activity of plant I remained somewhat more than twice that of plant II, indicating that potassium must play a rôle in the actual formation of invertase.

TABLE VIII

Effect of equalizing potassium in cane blades I and II upon invertase activity thereof

Lot	$KMnO_4$ determined (cc.)	N/10 $KMnO_4$ calculated (cc.)	Invert sugar (mg.)
Blank Na....	1.10	.....	.....
Blank K.....	1.05	.....	.....
I.....	40.50	39.211	63.6
II.....	18.85	17.714	28.2

IV. Moisture determinations

The results of the moisture determinations are presented in table IX, which shows that the amount of moisture in the sugar cane plants varied directly with the amount of potassium supplied.

V. SUGARS

The results of the sugar determinations are presented in table X. In every case plant II had the greatest percentage of sugars, and in general there is a negative correlation between the percentage of sugar and the amount of potassium supplied. The total amounts of sugar do not show a gradation because of the differences in the size of the plants.

TABLE IX

MOISTURE PERCENTAGES IN CANE

PLANT	TOPS, APRIL	BLADES, JUNE	STEMS AND SHEATHS, JUNE
I	82.4	73.2	83.2
II	77.7	70.3	77.8
III	79.3	69.5	79.5
IV	79.8	74.2	81.0

TABLE X

RESULTS OF SUGAR DETERMINATIONS

PLANT	REDUCING SUBSTANCES			SUCROSE			TOTAL SUGARS		
	Tops	Blades	Stems	Tops	Blades	Stems	Tops	Blades	Stems
	Percentages of sugar in sugar cane								
I	0.200	0.437	0.506	1.923	1.835	2.799	2.123	2.272	3.305
II	1.230	1.618	1.728	3.130	3.872	3.319	4.360	5.490	5.047
III	1.170	1.605	1.753	2.240	3.019	2.766	3.410	4.624	4.519
IV	0.858	1.299	1.074	2.276	1.924	2.987	3.134	3.223	4.061
	Total amounts of sugars in entire plants, expressed in gm.								
I	0.2210	0.3521	0.5617	2.1249	1.3946	3.1069	2.3459	1.7267	3.6686
II	0.3874	0.6472	0.4147	0.9859	1.5488	0.7966	1.3733	2.1960	1.2113
III	0.5967	1.0111	0.7450	1.1424	1.9020	1.1755	1.7391	2.9131	1.9205
IV	0.4693	1.2561	0.8732	1.2249	1.8605	2.4284	1.6942	3.1166	3.3016

In connection with the extraction for the sugar analyses, observations on the chlorophyll content were made. The alcoholic extracts of plants I and IV were a vivid pure green, while those of II and III were a yellow-olive green.

VI. Microchemical investigations

A. Leaves

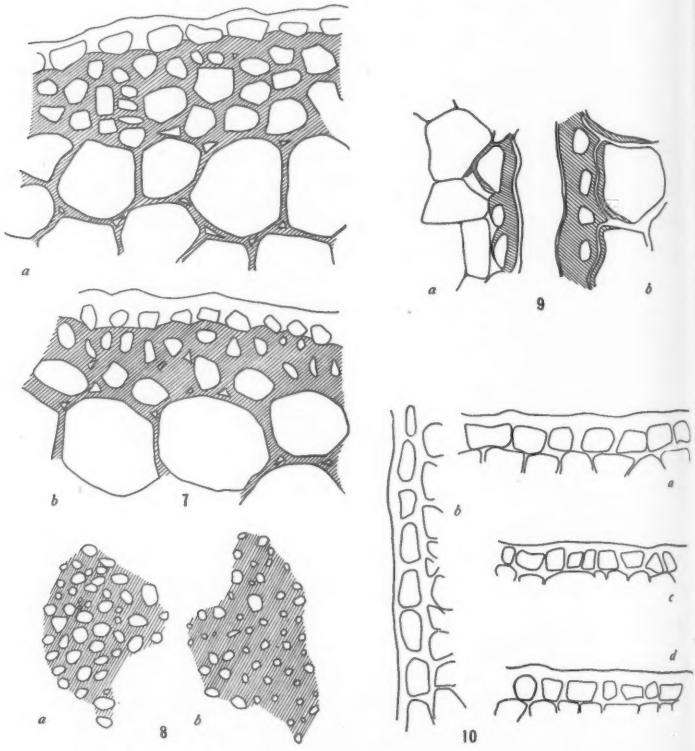
Tests on cellulose, lignin, calcium pectate, and cutin were made on cross-sections of equal-aged blades, the sections being made at about the middle of the leaf. There was no evidence that the amount of potassium affected the development of cellulose or calcium pectate, but an increase in cutin and a decrease in lignin were correlated with an increase in the amount of potassium in the nutrient solutions. The subepidermal plates composed of sclerenchyma cells, found just beneath the upper epidermis near the region of the midrib, and the ordinary girders of sclerenchyma found on the lower side of the leaf showed decided differences in the amount of lignin. Figs. 7 *a*, *b* and 8 *a*, *b* show that the sclerenchyma cells in the regions mentioned in plant I had larger lumina, and in general thinner walls than those in the potassium-deficient plants; but the differences in coloring, which cannot be depicted, were much more pronounced. Lignified walls of plants II and III stained a bright red while those of plant I were slightly orange-colored. Cutinized walls of plant I stained deeper red with Sudan III than did the walls of the other plants, which became yellowish red.

Sections cut through the sheath 1 inch from the blade showed that the potassium-deficient plants developed more lignin than those supplied with potassium (fig. 9 *a*, *b*).

B. Stems

Stems sectioned at the fourth internode differed in the amount of cutin and lignin and in the size of the cells. The cuticles of plants II and III were thinner than those of plants I and IV (fig. 10 *a*, *d*). The sclerenchyma walls were thinner and paler, and the lumina were larger in the bundle sheaths of plant I than plant II, when taken in the internode (fig. 11 *a*, *b*). The walls of vessels in similar locations were thinner in plants I and IV than in II and III (fig. 12 *a–d*). These results are not in accord with those of Purvis (**26**), who found the thickness of the xylem walls of *Dactylis glomerata* to be the same with and without potassium. As the diagrams in fig. 12 indicated variation in the size of the cells, several outlines of the vessels near

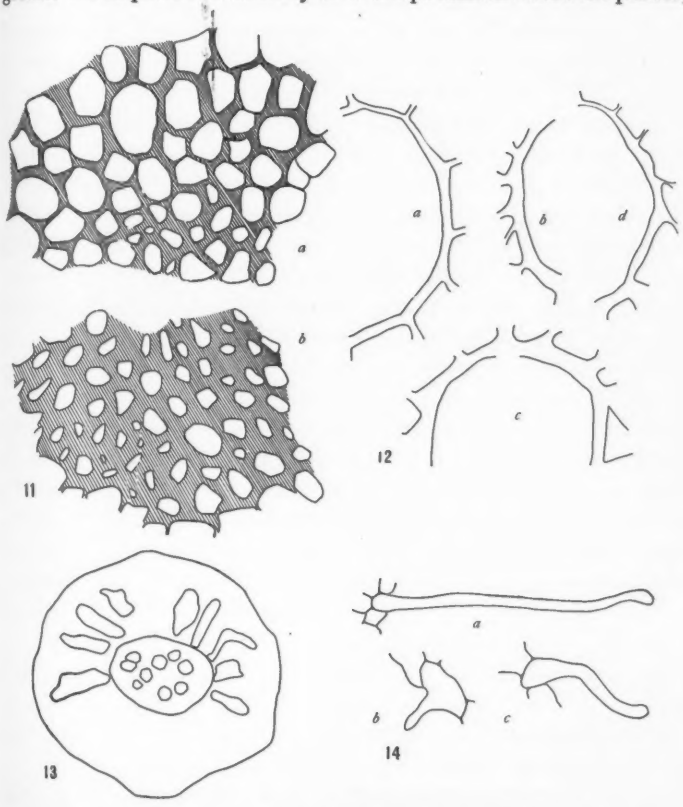
the center of the stems were made, which showed that the vessels of plant II were decidedly smaller than the others, while those of plant



FIGS. 7–10.—Fig. 7, effect of potassium on lignin; cross-sections of blades through upper epidermis showing subepidermal plates of sclerenchyma: *a*, plant I; *b*, plant II; ×35.5. Fig. 8, effect of potassium on lignin; girders of sclerenchyma under lower epidermis of blades, seen in cross-section: *a*, plant I; *b*, plant III; ×35.5. Fig. 9, effect of potassium on lignin; cross-sections of sheaths through lower epidermis: *a*, plant I; *b*, plant II; ×35.5. Fig. 10, effect of potassium on cutin; cross-sections of stems through fourth internode: *a*, plant I; *b*, plant II; *c*, plant III; *d*, plant IV; ×35.5.

III were only slightly smaller. A comparison of the largest parenchyma cells in the fourth internode showed a slight gradation in size. Besides these differences, certain other observations were made: the

plants receiving potassium had more wax in the wax bands; there was greater development of anthocyan in the potassium-deficient plants;



FIGS. 11–14.—Fig. 11, effect of potassium on lignin; cross-sections of stems through fourth internode, near center, showing portions of bundle sheaths next to phloem: *a*, plant I; *b*, plant II; ×35.5. Fig. 12, lignified walls of vessels near center of stems, cross-sections through fourth internode: *a*, plant I; *b*, plant II; *c*, plant III; *d*, plant IV; ×35.5. Fig. 13, cross-section of root of plant II, 1 inch from tip, showing cavities in cortex and irregular distribution of vessels in pith; ×6.0. Fig. 14, root hairs seen in cross-sections of roots 1 inch from tip: *a*, plant I; *b*, *c*, plant III; ×25.8.

and brown discolorations appeared in the bundles and the parenchyma of plant II.

c. Roots

Three striking abnormalities were found in plant II: the almost total lack of root hairs; the irregular distribution of the vessels in the pith (fig. 13); and the presence of large cavities in the cortex (fig. 13). The solid cortex of plant I contrasted sharply with that of plant II, which was full of cavities, possibly similar to the loose tissue noted by Sommer and Sorokin (31) in longitudinal sections of the root tips of *Pisum sativum* grown without potassium and with boron. The difference in the size of the hairs on plant I and III is depicted in fig. 14 *a–c*. The endodermis and the sclerenchyma of the stele of plant II stained more heavily than that of plant I, indicating a greater development of lignin, but no differences in the thickness of the walls were noticeable in the sections.

Discussion

The purpose of these experiments was not merely to grow plants with and without potassium and note differences, but to attempt to obtain a graded series correlated with the amounts of potassium in the nutrient solutions. The solutions contained the following amounts of potassium:

Solution	Per cent	P.P.M.
I	0.07038	703.8
II	0.00000	000.0
III	0.00039	3.9
IV	0.00390	39.0

An examination of the photographs and a study of these data show that positive gradations have been obtained in weight and certain other measurements of size, moisture percentage, invertase activity of blades, peptase activity of blades, stems plus sheaths, and roots, catalase activity, cutin and wax formation, size of cells, and production of root hairs. The size measurements which varied directly with the amount of potassium were weight of tops, blades, sheaths plus stems, roots, length of tops, leaf breadth, and stem girth. Plant IV surpassed plant I in several respects, for example, length of tops on June 2. This, although possibly due to the fact that plant I was pot-bound, indicates that the amount of potassium in solution IV (39 p.p.m.) was almost enough for usual growth. It seems, therefore,

that under these cultural conditions sugar cane plants require much less potassium for their usual physiological processes than the amount contained in SHIVE's best solution. This amount is much greater than that determined by other investigators as the minimum potassium requirement for the maximum growth of plants. JOHNSTON and HOAGLAND (**14**) found that a solution originally containing 4.9 p.p.m. potassium flowing at the rate of 8 cc. per minute per plant was as good for tomato plants as their control, 35.1 p.p.m. BARTHOLOMEW and JANSSEN (**2**) reported that about 2 p.p.m. is essential for the best growth of oats, cowpeas, soy beans, and cotton; while their maximum growth of sudan grass occurred at 3 p.p.m. PARKER and PIERRE (**24**) found that the minimum concentration of potassium for the maximum growth of corn and soy beans was 2 p.p.m., or possibly less. Since the results of the present investigation show that 3.9 p.p.m. potassium is decidedly insufficient for the best growth of sugar cane, one may conclude that cane requires more potassium than the other plants mentioned. This is in agreement with the statement made by RUSSELL (**29**) that sugar-producing plants stand most in need of potassium salts. One possible explanation of the greater need for potassium in sugar-producing plants is found in the evidence presented in this paper that potassium plays a rôle in the formation of invertase in sugar cane.

The plants, of course, all started with a reserve of potassium in the 2 inches of seed piece transplanted with them. However potassium functions in the plant, it seems reasonable to suppose that this reserve would last for a considerable time. As already stated, their growth rate remained equal until March. Four months seems a long time for their potassium reserve to be sufficient for their needs, the truth of which was evidenced by the ability of plant II to equal the others in size and appearance. This might be explained in two ways. Perhaps potassium is important as a catalyst, and as such is not rapidly used up but may act again and again; or perhaps another factor limited the growth of all the plants during those four months. Both possibilities might be true. Because gradation in growth and size was apparent after the days became longer and sunnier, it seems likely that light was the limiting factor. The results obtained in growing buckwheat and soy beans during the fall and spring indi-

cated the same possibility. Perhaps during the fall and winter all the plants had sufficient potassium for their growth processes, which went on slowly because of lack of light. In the spring light ceased to be the limiting factor, however, and the growth rate therefore increased in proportion to the amount of potassium supplied. These results agree with those of JOHNSTON and HOAGLAND (14), who found in their work with tomato plants that shade and low potassium are apparently two limiting factors which operate at the same time; and that the maximum yield would be obtained only by increasing both factors.

The potassium-deficient plants had longer roots than the others (tables I and III). Somehow there was a lack of differentiation and formation of secondary roots, so that evidently all the available food went into the lengthening of the main roots. Other workers have obtained similar results. MICHEELS (20) found that wheat plants supplied with sodium have a greater root length than those furnished with potassium. WALLACE and HUTCHINSON (34) found that the root systems of willow cuttings grown in a solution lacking potassium were more slender and had fewer secondary roots than those supplied with potassium. There are several possible explanations of these differences in root development. The greater percentage of sugars in the potassium-deficient plants may have resulted in the lengthening of the main roots, just as the tomato plants studied by REID (28) had a more vigorous development of roots when the carbohydrate reserve was high. The increase in the amount of calcium and phosphorus with potassium deficiency, as noted later in the work of JOHNSTON and HOAGLAND (14), may be another explanation, since these elements increase root length. REED (27) found that potassium is essential for mitosis; perhaps the cells of the pericycle did not have enough potassium for division to occur and therefore few secondary roots started. Or it may be, as suggested by MOLISCH (21), that potassium is important in building up the plasma of the growing points; there may not have been enough potassium in the plants for the development of many secondary growing points. This possible relationship of potassium to the formation of protoplasm in the growing points may be correlated with the activity of peptase, as discussed later. Probably not one but all of these factors contributed

to the greater length of the main roots and the lack of differentiation of branch roots in the potassium-deficient plants. What are the physico-chemical processes which result in the beginning of branch roots? Whatever they are, lack of potassium seems to upset the physiological balance, as few were seen by microscopic observation. The development of cavities in the roots of plant II, as seen in cross-section (fig. 13), may be due to the death of the cells or possibly to differences in the cell wall constituents. The occasional dropping out of entire rows of cells was noted by SOMMER and SOROKIN (31), in longitudinal sections of the root tips of *Pisum sativum* grown without potassium and with boron.

Table XI shows the enzyme results obtained. It should not be surprising that all enzymes are not affected in the same way. Life

TABLE XI

SUMMARY OF ENZYME RESULTS

ORGAN	DIASTASE	INVERTASE	PEPTASE	EREPTASE	CATALASE
Tops	−*	o*	o	o	+*
Stems and sheaths	−	o	+	o	+
Blades	−	+	+	o	+
Roots	o	.....	+	.....	o

* + = potassium increases action; − = potassium decreases action; o = potassium seems to have no effect; blank = not tested.

processes in a plant are so interwoven and concatenated that an upset of one reaction in one direction may result in derangements of many other reactions, and it may very well be that cause and effect can never be disentangled.

The diastase results presented in this paper are similar to those obtained by DOBY and HIBBARD (7) with the leaves of the sugar beet. They are also similar to the present writer's unpublished results with wheat and soy beans, and with buckwheat grown in the spring. Buckwheat grown in the fall and therefore with deficient light showed better diastase activity with the plants receiving potassium than with the potassium-deficient plants. The suggestion proposed by DOBY and HIBBARD that the greater diastase activity in the plants grown with insufficient potassium might be due to starvation, seemed not to explain the phenomenon fully. Since diastase is considered to be active in starch formation as well as digestion, and since

higher percentages of sugars were obtained in the potassium-deficient plants, is it not possible that the increased diastase activity of the plants starved for potassium is explainable by KNUDSON'S theory of the quantitative regulation of enzymes? KNUDSON (**16**), working with *Aspergillus niger* and *Penicillium* spp. grown on synthetic media, found that they did not form tannase, but after the addition of tannic or gallic acid tannase was formed. Since a greater amount of tannase was produced when there was a larger amount of tannic acid in the medium, it was suggested that the production of enzymes is regulated quantitatively by the amount of the substrate present. Other examples of the effect of the composition of the medium upon the development of enzymes are reviewed by WAKSMAN and DAVISON (**33**). In the present case the greater diastase activity of the potassium-deficient plants may have been caused by their increased percentage of sugars, which constitute the substrate necessary for the formation of starch by diastase.

TABLE XII

GROWTH FROM APRIL TO JUNE (ESTIMATED FROM DIFFERENT PLANTS)

PLANT	LEAF BREADTH (CM.)	STEM GIRTH (CM.)
I.....	1.3	2.3
II.....	0.3	1.5
III.....	0.6	1.6
IV.....	1.4	2.2

Possibly sugar cane resembles the tomato plants studied by JOHNSTON and HOAGLAND (**14**) in absorbing more phosphorus when the supply of potassium is deficient. If this is true for cane, possibly the increased amount of phosphorus is another factor in causing the greater diastase activity in the potassium-deficient plants, since phosphorus is known to favor that enzyme.

Diastase activity may be correlated with the growth rate. Subtracting the results in table I from those in table III, the data presented in table XII are obtained. The growth of the stems as shown by their girth from April to June decreased in this order: I, IV, III, II. Table IV shows the diastase activity of stems collected in June decreased in this order: II–III, IV, I. Table X shows the percentage

of total sugars in stems and sheaths decreased in the same order. Breadth of leaf decreased in the order: IV, I, III, II. Table IV shows the diastase activity of blades collected in June decreased as follows: II–III, I, IV. The percentage of total sugars in the blades decreased in the order: II, III, IV, I. In general, the more rapidly the plant grew, the less the diastase activity. Possibly the increased growth of the control plants used up the sugars in the formation of protoplasm, thus leaving no surplus sugars to increase the diastase activity by quantitative regulation; but lack of potassium in plants II and III curtailed their growth, which resulted in the accumulation of an excess of sugars, which in turn caused their greater diastase activity.

The weak diastase activity of all the roots was not surprising since the root does not manufacture starch. This, as well as the results with invertase, illustrates the fact that different organs of the same plant react differently.

The same organs react differently at different ages. Tops collected April 14 showed no effect of potassium upon invertase, but by June 2 the blades of plant I had developed a much greater activity while that of the other plants remained about the same. The activity of the stems plus sheaths also increased, but more uniformly than the blades, as the intermediate plants were the extremes in activity. Peptase is another illustration of the effect of age. Tops collected in April showed equal activity while all organs collected in June showed increased activity with increased potassium. External symptoms are undoubtedly the outward expression of internal derangements, some of which are at first too delicate for determination by our clumsy methods, but with increased age the unbalanced physiological conditions become more pronounced and hence are more readily determined.

The invertase results in the blades led to an attempt to equalize the potassium in the material, as explained in section III of the results. The blade materials contained different amounts of potassium which might be directly responsible for the results. It was thought that if equalizing the potassium resulted in equalizing the invertase activity, then potassium would be considered important as a coenzyme or regulator of invertase activity. But if equalizing the

potassium did not result in equal invertase activity, it would be concluded that potassium is somehow necessary in the actual formation of invertase. The results presented in table VIII certainly support the second theory, since the activity of plant I after the equalization of potassium remained somewhat more than twice that of plant II.

If the invertase of blades differs, why does not that of the stems? Why are the results in all the stems more like the results in the blades of plant I than plant II? Is it possible that the stems contain more potassium than the blades? Unfortunately these determinations could not be made.

Table VI shows that the activity of peptase was greater in the plants receiving potassium than in the potassium-deficient plants. This was even more noticeable in the roots than in the parts above ground. The deficient peptase activity would naturally cause a decrease in the formation of proteins and hence in the development of protoplasm. This seems an adequate explanation of the lack of development of secondary roots, the cessation of growth of the tops, the dieback of the leaves, and possibly even of the derangements in the formation of chlorophyll.

The importance of the catalase results depends upon the importance attached to catalase as an enzyme. MORGULIS (**22**) has claimed that it is not a measure of metabolic activity. However that may be, catalase activity was found to vary directly with the potassium supplied and the growth responses, except in the roots.

It has been shown previously that the enzyme response varies with the age of the plant and the organ studied. Other experiments not here reported have shown that it varies with different species of plants, studied in similar ways. The peptase activity of the blades of soy beans varied directly with the amount of potassium; there was no difference in the leaves of buckwheat at the age studied. Buckwheat leaves had greater activity of ereptase with increasing amounts of potassium, but soy bean blades showed decreased activity with greater potassium content. The catalase activity of buckwheat varied directly, that of soy bean blades varied inversely, while that of wheat seemed unaffected by the amount of potassium supplied. It is evident that a deficiency in potassium upsets the balance of physiological processes governed by enzymes. It seems more reason-

able to expect that the balance in different plants would be upset differently, than that all plants would react in the same way.

Added to the direct effect of potassium deficiency in causing decreased synthesis of proteins, cessation of growth, etc., there may be an indirect effect in the influence of the lack of potassium upon the intake of other ions. JOHNSTON and HOAGLAND (14) found that tomato plants grown with low potassium content in the nutrient solution absorbed greater amounts of calcium, magnesium, and phosphorus than plants supplied with the full amount of potassium. Undoubtedly the increase in these three ions influences the physicochemistry of the potassium-deficient plants, if this increase is characteristic of all kinds of plants grown with a small amount of potassium. Possibly the greater length of the roots of the plants starved for potassium was influenced by the increased amounts of calcium and phosphorus, since these elements are known to increase the length of roots. The phosphate ion is known to be favorable to diastase activity, since the normal transformations of starch are somewhat dependent upon phosphorus. This may be one factor in influencing the greater diastase activity of the potassium-deficient plants. An increase in the magnesium content might be detrimental since magnesium is known to be toxic at times. The influence of all the essential elements must therefore be considered when studying the effect of the absence of one mineral nutrient. It may be that the problem of the rôle of potassium will be solved only after the equalization of all the other ions within the tissues of the plant.

The larger moisture percentage of the plants supplied with potassium (table IX) is perhaps due to their larger root system, or possibly to differences in colloidal water-holding capacity. It may also have resulted from their growth rates, since increased water content usually accompanies more rapid growth. Whatever the cause, it certainly is correlated with the amount of lignin and cutin. Deficient water supply in a cell would produce conditions favorable for condensation and hence elaboration of lignin; or the lower moisture content of the samples of the potassium-deficient plants may merely have resulted from the presence of more lignin in the samples. Transpiration from plants II and III may have been greater than from I and IV because of the decreased cutin in the former. The influence of potassium in

governing the turgor of plants has been mentioned by COPELAND (4) and by WEEVERS (35).

The decreased water content of the potassium-deficient plants may help to explain why plants starved for potassium sometimes show less resistance to frost than do plants supplied with potassium. The statement that potassium protects plants against frost has been made by LOEW (19), by PATTERSON (25), and by other workers, although the exact way in which it acts in this respect has not been definitely determined. The winter killing of plants is generally considered to be due to drying as well as to freezing, and it stands to reason that, other things being equal, plants with a lower water content would dry out more rapidly, especially when their cuticle is not well developed. Another factor involved in the resistance of plants to low temperatures is the nature of their proteins. HARVEY (10) found that certain changes in proteins occur, which are partly hydrolytic and which result in making them less easily coagulated. Perhaps peptase is one of the enzymes needed to catalyze these changes, which would therefore take place more slowly in the potassium-deficient plants. In short, potassium may increase resistance to cold by giving plants a greater water-holding capacity, a better developed cuticle which resists drying out, and more active peptase which is important in catalyzing certain changes in proteins in the process of the hardening of plants.

The sugar analyses showed that the potassium-deficient plants had a greater percentage of total sugars and reducing sugars than the plants with a larger amount of potassium. Table X shows a regular gradation; the less the potassium, the more the sugar. Sucrose gave a similar gradation except in the stems (table X), where, however, plant II had the largest amount. It is interesting that in the stems neither the sucrose content nor the invertase activity varied greatly, while in the blades the largest sucrose content was found in the plants having the least invertase activity. Does the theory of the quantitative regulation of enzymes fail here; or was the potassium content of plant II insufficient to produce much invertase? Perhaps the abundance of hexose may be responsible for the large amount of sucrose, because a great quantity of the sub-

strate may result in the formation of a large amount of the end product even though the enzyme is not very active.

Why do the potassium-deficient plants contain more sugar than the controls? It might presumably be due to the decreased anabolic or increased catabolic activity of the enzymes, which build sugars into higher compounds, or the increased anabolic activity in the enzymes of photosynthesis, or a combination of these. It will be remembered that peptase showed greater activity with increased potassium content. Perhaps the decreased protein synthesis due to the deficient activity of the enzyme peptase may explain the piling up of the sugars in the potassium-deficient plants. It would also explain their decreased growth. This piling up of carbohydrates with poor nutrition has been found by other workers. KRAYBILL stated in an address to the Botany Club of the University of Chicago (unpublished) that he has found increased carbohydrates with decreased nitrogen, sulphur, and phosphorus. HARTWELL (9) found increased starch with decreased potassium and other factors which inhibit growth. JANSSEN and BARTHOLOMEW (12), working with tomato plants, found that high nitrogen and high sugars were correlated in the blooming stage of the low potassium plants, and thought that this might be due to a lack of condensation of these compounds to more complex forms. There is need, therefore, for more work with enzymes. A quantitative measurement of peptase, with the potassium equalized as in the invertase work, might prove suggestive.

Another possible explanation of the accumulation of sugars in the potassium-deficient plants is that proposed by JOHNSTON and DORE (13) in connection with their studies of boron deficiency. They found that tomato plants starved for boron had a greater sugar content in the leaves than the control plants; and since they found phloem necrosis in the petioles and stems of the boron-deficient plants, they thought that the broken-down conducting tissues might be responsible for the accumulation of carbohydrates. Since phloem contains living protoplasm, it might suffer from any severe starvation. In the present investigation only slight evidence of derangements of the phloem was found in the potassium-deficient sugar cane plants: the occasional reddish discoloration of some of

the cells of the phloem in the leaves, and the brown discolorations in the bundles of the stems.

Since the potassium-deficient plants have larger percentages of sugars than the controls, one might hastily conclude that greater sugar production would result from starvation for potassium. Table X shows this to be erroneous. While the percentages vary inversely with the amount of potassium, the total amounts vary directly since the control plants are the largest in size.

The increased sugar content of the potassium-deficient plants may help to explain why they are more susceptible to disease. It may also cause their greater development of anthocyan.

Early workers claimed that potassium is essential for photosynthesis. Do these results necessarily disagree? If the percentage of sugars present at a given time may be used as a measure of photosynthesis, then it would certainly seem that the potassium-deficient plants carry on that process better than the plants supplied with potassium. But it has already been shown that decreased peptase activity accompanies deficiency in potassium and may be responsible for the accumulation of sugars. The piling up of sugars might conceivably result in a decrease in photosynthesis, since the accumulation of end products is known to decrease the rate of catalytic reactions. This is not essential to explain the decreased growth, since the deficiency in the protein enzymes could account for that. The results reported in this paper, therefore, neither prove nor disprove the theory that potassium is essential for photosynthesis. That increased sunlight hastened the growth of the plants supplied with potassium, but not those starved for potassium, might be indirect evidence in favor of the theory. More facts regarding the chemical nature of the photosynthetic process are needed before the effect of potassium can be studied directly. The results certainly support the theory that potassium is needed in the synthesis of proteins.

In general, the effect of the lack of potassium seems to be noncoordination, since the action of some enzymes is intensified and of others lessened; but one must not conclude that the function of potassium is the coordination of enzymes without first studying the effects of other essential elements upon enzymes. Lack of potassium stops growth, causes dieback, and decreases chlorophyll formation.

It may be that the cessation of growth is caused by a decrease in the manufacture of proteins, since deficiency in potassium has been shown to interfere with the activity of peptase. Perhaps the decreased formation of proteins results in the accumulation of sugars, which in turn causes increased diastase activity due to the quantitative regulation of enzymes. This increased diastase activity may indicate a possible increase in the activity of certain other enzymes which lead to the formation of lignin. Greater amount of lignin, poorly developed root system, differences in colloidal water-holding capacity, and other factors would lead to a decreased moisture content. This insufficient supply of water together with the deficient protein formation may explain the dieback of the leaves. Possibly the decreased activity of peptase is important also in explaining the underdevelopment of the secondary roots, and it may even explain the derangement in the formation of chlorophyll. If these derangements of the physiological processes in sugar cane, which have been indicated in the data here discussed, may be thus linked together in a chain of cause and effect, then they present a plausible explanation of the symptoms of potassium starvation.

Summary and conclusions

1. This paper deals with the effect of varying amounts of potassium upon the growth, enzyme activity, moisture percentage, sugar content, cellular structure, and microchemistry of sugar cane.

2. Sugar cane started from cuttings in October and transplanted in November began to show symptoms of potassium starvation in March.

3. The symptoms of potassium starvation obtained were decreased growth, dieback, and deficient development of chlorophyll.

4. It is possible to secure a gradation in growth correlated with the gradation in the amount of potassium supplied.

5. The size measurements which varied directly with the amount of potassium were: weight of tops, blades, sheaths plus stems, roots, length of tops, leaf breadth, and stem girth. The potassium-deficient plants had longer roots than those supplied with potassium.

6. The amount of potassium needed by sugar cane for its usual

physiological processes is less than that contained in SHIVE'S best solution.

7. An amount of 3.9 p.p.m. was found insufficient for the best growth of sugar cane.

8. Quantitative determinations were made of the activity of diastase, invertase, peptase, ereptase, and catalase of tops, blades, sheaths plus stems, and roots.

9. Potassium may increase or decrease enzyme activity or have no effect thereon.

10. The effect of potassium upon enzyme activity varies with the kind, age, and the organ of the plant studied.

11. The potassium-deficient plants had greatest diastase activity in tops, sheaths plus stems, and blades. The activity in the roots was equal in all the plants.

12. The invertase activity in the blades was greater in the control than in the potassium-deficient plants. The activity of the stems plus sheaths was equal.

13. The peptase activity of the blades, sheaths plus stems, and roots was greatest in the controls.

14. The ereptase activity was the same in all the plants.

15. The catalase activity was greater in the plants supplied with potassium, with the exception of the roots, in which the activity was equal.

16. Since equalizing the potassium content of cane blades does not equalize the invertase activity thereof, it seems likely that potassium may play a rôle in the formation of that enzyme.

17. The tops of the potassium-deficient plants had a smaller moisture content than the controls.

18. The potassium-deficient plants had greater percentages of total sugars, reducing sugars, and sucrose than the plants supplied with potassium.

19. Greater lignification occurred in the potassium-deficient plants, while greater cutinization was found in the plants supplied with potassium.

20. Abnormal distribution of vessels in the pith of the roots, small size of the vessels and the parenchyma cells of the stems, large

cavities in the cortex of the roots, and underdevelopment of the root hairs were found to attend the lack of potassium.

21. Suggestions are made regarding the causes of the symptoms of potassium starvation, as well as the greater resistance toward low temperatures and diseases found among plants supplied with potassium.

22. These derangements in the morphology and the physiology of sugar cane must be concatenated and an attempt is made to link them together in a chain of cause and effect.

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STAMINATE FLOWER OF ECHINOCYSTIS LOBATA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 396

WARD L. MILLER

(WITH PLATES XIII–XVI)

Introduction

For a long time the interest of botanists and also their ability to interpret have been challenged by at least three morphological situations in the Cucurbitaceae: (1) the epigynous character and the method by which the pistillate flower develops it; (2) the stamen situation which involves some stamens apparently complete and others apparently only half complete; and (3) the taxonomic position of the family which must be determined by the balance, or lack of it, between family characters peculiar to the Sympetalae and those peculiar to the Archichlamydeae. One of these situations, the second, has for nearly a century been a subject of controversy that has been intense and at times almost bitter; and that the matter is still in dispute is evidenced by the recent work and conclusions of HEIMLICH (6) on the subject.

The present investigation of the staminate flower of *Echinocystis lobata* has revealed a number of morphological details which are significant in that they contribute to the interpretation of all three of these situations; to the first indirectly, but to the others directly. An account of these details and of their significance is therefore timely.

Controversy over the interpretation of stamens in the Cucurbitaceae must have begun soon after the first taxonomic description of the family appeared in print, for even the more important formal papers on the subject date back as far as 1855, when NAUDIN (18, 19) expressed his conviction that the large tetrasporangiate stamen was normal, while the smaller bisporangiate one had resulted either from the abortion of the missing half or else from the splitting of a large stamen into two. Two years later PAYER (20) presented the opposing view, that the small bisporangiate stamen was normal, while the large tetrasporangiate stamen had resulted from the union of two

normal ones. Not until 1875 did the subject come prominently before the botanical public again, when both EICHLER (4) and VAN TIEGHEM (24) published their accounts of the stamen in this family. Both investigators looked upon the tetrasporangiate organ as being double (VAN TIEGHEM uses the expression "cinq termes doubles" as composing the typical androecium), although they disagreed over the method by which doubling might have occurred. Three years later BAILLON'S paper (1), which had been read before the French Association, was published. For NAUDIN and his supporters this paper contained an emphatic criticism, followed by the author's own interpretation of cucurbitaceous stamens, the latter based for the most part on his investigation of *Bryonia*, and essentially in accord with the interpretation of PAYER. BAILLON referred to the work of DUCHARTRE and to that of DECAISNE, both of whom agreed with NAUDIN, although DECAISNE was obviously ambiguous in many of his statements; he referred as well to TISON whose work on *Thladiantha* bore out his own conclusions. Again in 1886 BAILLON'S position on the stamen situation was published (2). MOREAU (17) reported briefly on an investigation of numerical variations among floral parts of *Bryonia* in 1914. The most recent morphological contribution has been made by HEIMLICH (6), who describes the androecium of *Cucumis sativus* as consisting of two complete stamens and one half-stamen, his interpretation therefore reaffirming the older one of NAUDIN.

Most of the recent work with the Cucurbitaceae has been done, not on floral development, but on cytological aspects of sporogenesis, pollen tube development, and embryology. CASTETTER (3), HEIMLICH (7), KIRKWOOD (10), and KOZHUKHOV (11) have investigated microsporogenesis; while KRATZER (12), LONGO (14), and Miss TILLMAN (23) have worked on various features of the embryo sac and on development of the seed. The pollen tube has received attention from KIRKWOOD (9), LLOYD (13), and LONGO (15). Both KIRKWOOD (8) and LONGO (16) have made embryological studies, and HAGEDOORN (5) has contributed a paper on parthenogenesis. And to these morphological contributions might well be added the two papers of TIEDJENS (21, 22) on sex ratios and on the effects of environment on fruits.

Investigation

Materials

Staminate inflorescences of *Echinocystis lobata* were collected in the summer of 1927 at Grove City, Pennsylvania, and were fixed in formalin-acetic-alcohol. Young inflorescences were fixed without dissection at all, but from the older ones flowers were isolated and treated individually. The long sepals and petals were cut away from most of the mature flowers, so as to avoid the entrapping of air bubbles and to facilitate fixing, infiltration, and general handling.

Sections 5 μ in thickness were used. Part of these were stained with iron-haematoxylin and part with a safranin light-green combination. Safranin was used in 50 per cent alcohol while light-green was used in 95 per cent alcohol. After sections had stood in safranin they were transferred to tap water, where they were left for 10–15 hours before being run up to the light-green.

Mature flower

The broad and somewhat flat staminate flower of *Echinocystis lobata* has its receptacle cup, which is commonly called the calyx, extending upward to about two-thirds or three-fourths the length of the short androecium. The receptacle bears only a few scattered hairs on its outer surface and these are mostly non-glandular, but its inner surface is densely covered by short, multicellular, glandular hairs. The sepals are six in number, long, slender, acute, and wide spreading. They are sparsely covered, chiefly at the basal ends, by hairs which are multicellular and non-glandular. The petals, which are also six or rarely seven in number, alternate with the sepals. Unlike the latter, the petals are flat and ribbon-shaped, rounded at the tips and white in color. Although they too are long, they spread less widely than the sepals, and near their juncture with the receptacle cup they come in close contact with one another by their edges, thus forming the short corolla tube. On both their surfaces the petals bear simple multicellular hairs, many of which seem to be glandular, although the terminal cells are but slightly if at all enlarged.

The androecium is a complex structure, almost spherical in shape excepting for its two short basal stalks. Its surface is lobed and strongly contoured by reason of the six S-shaped microsporangia

(fig. 1), which ultimately form the three locules. If, as is commonly done, the number of stalks and of locules be taken as indicators of the number of stamens present, then the flower has two stamens, one with two locules (four sporangia) and the other with but one (two sporangia). Both stamens adhere to each other by the inner faces of their connectives, however, so that, above the two short stalks, the whole androecium seems to be a single fleshy organ.

Origin of floral parts

The flower is first recognizable as a hemispherical mass of meristematic tissue (fig. 2). From the very first, however, the outermost cell layer is differentiated enough to be visibly distinct, and it is continuous with the epidermis of that cauline member of the inflorescence which bears the flower. In the inflorescence there are neither leaves nor bracts which subtend individual flowers. Very early the shape of the flower primordium changes from that of a hemisphere to that of an inverted, truncated cone whose truncated peak is to become the flower pedicel, and whose base is to become the receptacle, giving origin to perianth and stamens. The base of the inverted cone, due to an upgrowth of its circular margin, becomes slightly concave (fig. 3) and subsequently cup-shaped, with six marginal, hemispherical swellings. The latter are to develop finally into the six sepals. Almost immediately the petal primordia appear, six (or rarely seven) in number, alternating with the sepal primordia, and projecting from the inner surface of the cup toward the flower center (fig. 4). While the juncture of petals and receptacle is actually at a lower level than that of sepals and receptacle, it is morphologically at a higher level, since it is the inner one of the two levels and therefore nearer the tip of the floral axis.

After the perianth has thus started, the stamens appear. These, like both sepals and petals, are first recognizable as dome-shaped swellings of meristematic tissue on the inner surface of the concave receptacle. They are nearer the floral axis than are the perianth members, however, and from the first are of noticeably greater diameter (figs. 5, 6). Although the mature flower has what seem to be but two stamens, the embryonic flower has three separate stamen primordia (fig. 8). Two of these arise close together on the same side

of the flower, while the third is opposite them and about equidistant from both. Growth of these primordia is so rapid that the first relative distances between them are soon altered, and they come later to occupy positions on the receptacle floor almost equidistant from one another. This change occurs so early that it is impossible to determine from position alone what the exact arrangement of stamens is, relative to that of petals or of sepals. And neither can their arrangement be determined, in the staminate flower, from their vascular supply, because here, as will be shown later, there is no constant association between stamen bundles and perianth bundles.

The situation in the pistillate flower is sufficiently different from that in the staminate, however, to constitute a clue which seems to determine the matter of stamen arrangement. The pistillate flower has three staminodia which arise exactly as do the stamens in the staminate flower, that is, two close together and the third opposite them around the circumference of the receptacle base. Since these organs always remain small while the rest of the flower is growing, the danger of their being crowded out of original positions is much reduced compared with that in the other flower. Moreover each staminodium is supplied by a single bundle, which, in the part of the receptacle that has grown up around the ovary, is distinctly associated with the vascular supply of a perianth member. Here then is the kind of evidence that can determine the question of arrangement for the pistillate flower; if this evidence can equally well be applied to the problem in the staminate flower, that problem too can be solved. In the pistillate flower the two staminodia which arise close together can be traced by their vascular supply to two bundles which supply adjoining perianth members of different cycles (petal and sepal). The third staminodium, from the same kind of evidence, lies opposite another petal. This evidence indicates that the three staminodium primordia are members of two different cycles; two, lying opposite the petals, are in an upper cycle, while the third, lying opposite a sepal, is in a lower one.

The presence of staminodia in the pistillate flower and the presence of a pistillodium in the staminate flower of other genera (for example, *Cucumis*), would lead one to anticipate a pistillodium in the staminate flower of *Echinocystis lobata*, but none occurs. There

are no visible evidences at all of carpels in the staminate material available for this study.

Receptacle

Activity in the receptacle does not cease with the mere production of the primordia of floral leaves and stamens, for no sooner are these organs well started than the receptacle elongates noticeably, carrying sepals and petals up and over the young stamens (figs. 7, 11, 13, 15). It is of some significance whether this elongation results from activity of a limited zone which retains its original meristematic properties, from relatively unrestricted cell division throughout the entire receptacle, from cell enlargement alone, or from some combination of these alternatives; for, among the host of perigynous and epigynous flowers, there is doubtless considerable variation in this feature which may have phylogenetic, ecological, and (in pistillate flowers) even economic importance. In the case of *Echinocystis* the early elongation of the receptacle, up to the stage represented by fig. 15, is due almost exclusively to cell division in that part of the organ between stamen and petal bases. This is evidenced not only by the fact that receptacle cells of a half-grown flower are more numerous and but slightly larger than those of a young flower, but also by the fact that mitotic figures are numerous in the material (figs. 16, 17, 18). The figures most frequently met are those of transverse divisions (fig. 16) which result in elongation of the receptacle. A few are longitudinal (fig. 17), however, resulting in increased thickness, and a few more are tangential, resulting in increased circumference. Mitotic activity, rather than being restricted to a limited meristem or cambium, is general throughout that part of the receptacle outside the stamen cycles; and up to the time of complete cessation of figures the latter are equally numerous in both upper and lower levels.

The decline and final cessation of cell division in the receptacle, however, does not mark the end of that organ's growth; in fact, the receptacle may be no more than half-grown when mitosis stops. Subsequent to that time elongation is a matter of cell growth only (figs. 18, 19). Elongation in this second phase is no more localized than were the mitoses which preceded it, for cells at both high and low levels contribute to it. When mature the receptacle extends up about

three-fourths the length of the stamens, constituting the so-called "calyx cup."

As the receptacle approaches maturity it becomes spongy in texture, due to the development of schizogenous, intercellular spaces (fig. 20). Such spaces are more extensive in the upper part of the organ than they are in the lower, and their gaseous contents are continuous with the external atmosphere through numerous stomata (fig. 21), which are irregularly scattered over the outer surface. No stomata have been seen on the inner surface. Chloroplasts are present in parenchyma cells throughout the receptacle, but as a rule none occurs in the epidermis.

Epidermal hairs are numerous on the receptacle, particularly over its inner surface. Here the hairs are simple, multicellular, and glandular, the glands consisting of one, two, or several cells (figs. 22, 23). Hairs with multicellular glands are confined to the lower third of the receptacle cup, while those with one- or two-celled glands are scattered, without other limitations than that they are more numerous over the upper part. It is characteristic of all glands above the lower extremities of the anthers that they contain an abundance of coarse granules which stain deeply with safranin (fig. 22). No microchemical tests were made to determine the nature of these granules, but it is probable that they represent one or more secretion products in their pre-secretion phases. It is equally characteristic of all glands below the anthers that they lack these conspicuous granules. Their large nuclei and their densely staining cytoplasm, however, strongly indicate their secretory function (fig. 23). On the outer surface of the receptacle hairs are much less numerous than on the inner surface. What few hairs do occur are simple and mostly non-glandular (fig. 24), although now and then one finds a glandular hair similar to those high up on the inner receptacle surface.

Twelve vascular bundles traverse the receptacle cup vertically. No well defined branches come from any of these twelve bundles excepting in the upper part of the receptacle, and even then not all of the twelve branch. Here and there groups of elongated cells diverge out from a bundle for a short distance, but invariably the cells contain protoplasm and are differentiated in almost no other feature than their dimensional proportions and their position. Near the up-

per limit of the receptacle, just below the level where sepals and petals become distinct, six alternating bundles of the total twelve branch in a very definite fashion, giving rise to bundles that supply the perianth members as will be described later. Here also a very few of the vertical bundles may be connected in twos by slender, oblique vascular strands.

Calyx

All six of the sepals are separate throughout their entire length, and therefore there is no real calyx cup. As in the receptacle so in the sepal, elongation results at first from cell division (figs. 25, 26), and finally from cell growth (fig. 27). There are two important differences, however, between the elongation of the receptacle and that of a sepal. In the first place mitotic figures in a young sepal are prevailingly those of transverse divisions, while longitudinal and tangential divisions are exceedingly rare. As a consequence the sepals grow up into long, slender, widely divergent organs, ranging in their cross-sections from elliptical through triangular to circular. In the second place mitotic activity continues for a much shorter time in a sepal than in the receptacle, so that the former's growth is almost exclusively the result of cell enlargement. Here, as also in the receptacle, both mitosis and cell growth are general in their distribution; there is no restricted meristem.

All cells of the epidermis and of the inclosed chlorenchyma are large, and their cytoplasm, due to the development of large vacuoles, becomes very diffuse. The epidermis is thinly cutinized and is broken on the under surface by scattered stomata. While hairs are borne on both upper and lower surfaces they are few in number, and are mostly confined to the proximal or basal portions of the sepals. In structure they resemble the external hairs of the receptacle (figs. 22, 24).

At the base of a sepal the chlorenchyma exhibits the same spongy structure as does that of the receptacle; but out away from the base the intercellular spaces diminish progressively until, at the distal end of the organ, they are nothing more than mere breaks at the cell corners. All parenchyma cells between epidermis and vascular tissues are chlorophyll-bearing.

A single vascular bundle enters each sepal from the receptacle and traverses its entire length. Infrequently this bundle branches

and then the two resulting bundles extend to the sepal tip. Xylem is visibly differentiated to within one cell layer of the epidermis at the sepal tip, but visible differentiation of the phloem ceases somewhat farther back than that.

Corolla

Both mitosis and cell enlargement are responsible for the growth of corolla tube and corolla lobes, although it is not until near their maturity that cell enlargement becomes a noticeable factor (figs. 28–31). Long after cell division has ceased in the sepals it continues in the corolla. The majority of mitotic figures are those of transverse divisions, but many are longitudinal and many others tangential.

It has been commonly accepted that the corolla lobes of sympetalous flowers have their origin as has been described for *Echinocystis*, and that the corolla tube develops only after meristematic activity is resumed in the receptacle immediately below the lobe bases. Such a resumption of activity would cause the whole region to elongate as a cylinder, bearing the separate lobes on top. While this method of growth may apply to many, or even to most, sympetalous flowers, it hardly applies here. There is of course a corolla tube, but it does not possess the smooth continuity that would result from a continuous ringlike meristem at the juncture of corolla and receptacle. Rather it is a tube composed of individual vertical units, clearly marked off from one another throughout most of their length, but nevertheless grown together by their adjacent edges firmly enough to form a weak tube. The vertical units are of course the six petals. Each is so wide that it rubs shoulders, so to speak, with each of its two neighbors, and, since all of them develop in unison, there is in a sense a unit growth of a cylinder. At the same time, however, each petal is completely surrounded by its own epidermis, and certainly possesses its own individuality. There is no difficulty in tracing the epidermal layers of adjacent petals up through the "joint" where they are in contact (fig. 32), excepting in the middle region of the tube where papillate epidermal cells of each petal invade intercellular intervals in the other (figs. 33, 34). At the upper end of the corolla tube the petals diverge not suddenly but very gradually from one another (fig. 35), each with papillate epidermal cells which correspond with epidermal intervals in the other. The corolla tube then

is composed of members which, although adherent without being fused by cell fusions, are nevertheless distinct.

The epidermis of the petals is not cutinized. It bears hairs on both outer and inner surfaces, all hairs being simple and multicellular and probably glandular, although the terminal glandular cell in every case lacks the large granules which occur in the receptacle glands (fig. 36). The parenchyma is spongy throughout (fig. 31), and, for a short distance up from the receptacle, is chlorophyll-bearing. No stomata have been seen in the epidermis of petals.

Each petal is traversed by five vascular bundles, all of which enter from the receptacle. None of these bundles is branched excepting near the distal end of a petal, and even there branching is not frequent. Not far behind the petal tip each of the two marginal bundles connects with its nearest neighbor, which lies about midway between itself and the median bundle of the petal (fig. 42). This connection may be direct, in which case the marginal bundle itself anastomoses with its neighbor, or it may be indirect, in which case the marginal bundle is connected by a branch with the neighboring one. In the latter event the marginal bundle extends but little nearer the petal tip, where it ends blindly.

Stamens

Soon after the rise of the three stamen primordia (fig. 8), meristematic activity is resumed in the periphery of the receptacle between those two which are nearest together, and from then on the two original primordia thus involved develop as a single bilobed organ (figs. 10, 12). The third primordium develops independently. It follows, therefore, that, although three stamens have their start in the embryonic flower, there will be only two distinguishable ones in the mature flower. It is obvious that the two mature organs cannot be homologous, for while one is a simple stamen, the other, being made up of two simple ones, is compound. It needs to be emphasized that the compound stamen has resulted not from a fusing of two separate members, but from two members which, possessing somewhat of their respective individualities, have grown up almost in unison without having ever been entirely separate.

After elongating slightly the stamens begin to lobe, developing at

their free ends rounded protrusions which form right angles with their main axes (figs. 11, 13). As would be expected, the compound stamen develops twice as many main lobes as does the simple stamen (fig. 14). When seen in section, however, the subdivision of the main lobes will seem to vary, depending upon the vertical level from which the section is taken.

The two stamens maintain their separate identities for some time, but after a while even these are lost to the superficial observer; for as the stamens become thicker and as their inner faces are forced together throughout all but the basal part of their length, they come to have the appearance of one organ with two stalks (fig. 15). Since considerable growth and adjustment occur after such contact is effected, the two organs become strongly adherent to each other, papillate epidermal cells of the one dovetailing with similar cells of the other (fig. 37). Close as this union may be, however, no cases have been seen of cell fusions.

At about the time that the stamens begin to lobe the sporogenous tissue becomes differentiated. At first the subepidermal tissue to a depth of four or five cells is dense and deeply staining, and all of these cells have essentially the same appearance. Soon thereafter some of the cells of the outermost hypodermal layers cease to divide as rapidly as their neighbors and become conspicuously larger; thus differentiated, they constitute the archesporial tissue. This tissue occurs in two rows which represent the two microsporangia, and they are so arranged about the stamen as to describe a much compressed letter S. The small stamen develops but one of these double rows, or two sporangia, while the large stamen develops two double rows, or four sporangia (fig. 1). In the tetrasporangiate stamen each pair of S-shaped sporangia is so oriented that its lower end is near the flower center and its upper end is away from the center and toward the circumference. In the bisporangiate stamen the plane of the S is parallel with a cord of the floral circumference, and hence neither of its ends is nearer the flower center than the other.

All of the archesporial cells divide periclinally, each original S-shaped row thus forming two rows, one outer and one inner. The outer one is immediately beneath the stamen epidermis and is the primary parietal row, while the inner one is next inside the primary

parietal row and is the primary sporogenous row. The primary parietal cells, by dividing longitudinally, transversely, and tangentially, develop a three-layered sporangium wall under the stamen epidermis (figs. 38–40), the innermost of which becomes the tapetum. While most of the primary sporogenous cells grow directly into large microspore mother cells without further division, a few divide once or twice; as a consequence the row of sporogenous cells, originally one cell thick throughout, becomes two or three cells thick here and there in its course. The mother cells round off and divide twice, each forming four microspores. The latter are held together for a time in a mucilaginous matrix which forms, apparently, from some of the mother cell cytoplasm that does not participate directly in forming the four spores.

As the stamens mature, their connectives become highly spongy by reason of the formation of large schizo-lysigenous cavities. The growth pressure to which the contiguous faces of the stamen connectives are subjected, plus the disintegration of subepidermal cells, together serve completely to obliterate, here and there, the visible boundary between the stamens as they are observed in section. However, throughout most of the boundary region the two limiting epidermal layers are distinct, even in mature stamens. And even if both epidermal layers cannot always be distinguished along the boundary, in most cases at least their location is marked by a sheet of collapsed and distorted cells which take a deeper stain than do the fragmentary neighboring tissues.

Each stamen, whether tetrasporangiate or bisporangiate, is traversed by a single bundle which enters from the receptacle. Neither bundle branches excepting near the distal end of the organ, but here the bundle in the small stamen has one branch which extends toward the coiled sporangia, while that in the large stamen has two branches (fig. 43).

Vascular continuity

The flower pedicel is traversed by three vascular bundles. Where the pedicel broadens out into the receptacle these bundles break up into numerous, short, anastomosing branches which form a low dome-shaped tangle of vascular elements. From this tangle, through

which it is impossible to trace any single course, twelve bundles ascend obliquely through the receptacle and supply the perianth. As shown in fig. 41, however, a few of the twelve have indirect rather than direct connection with the vascular dome, for they are but branches from other bundles which do rise directly from the dome.

In the upper reaches of the receptacle cup six alternating bundles of the twelve are branched in a very definite manner (fig. 42). Each of the six branched bundles has two lateral (tangential) branches, beyond which it continues its course up and out into a sepal. Each of the two side branches continues out into a petal on the corresponding side. Just above the origins of these two branches they themselves are connected by another short bundle, oriented horizontally, and from the last there arise two more which extend out as marginal bundles into corresponding petals. The six bundles of the receptacle which do not branch before leaving it extend directly out into the six petals, each bundle occupying the median position. Each sepal therefore is supplied with a single bundle which either may or may not branch after leaving the receptacle. And each petal is supplied with five bundles, the middle one of which extends out directly from the receptacle base, right and left intermediate ones of which arise as branches from right and left sepal bundles respectively, and marginal ones of which arise as branches from the short, horizontal connecting bundle that unites the two branches of a sepal bundle.

Each stamen is supplied by a single bundle which extends upward directly from the vascular dome at the base of the receptacle (fig. 43). No constant association between these two stamen bundles and those supplying the perianth could be discerned, although such association does exist in the pistillate flower, as already described.

Discussion

Three matters having considerable morphological significance stand out prominently from the foregoing detail: (1) the unit individuality of the bisporangiate stamen; (2) the occurrence of stamens in two distinct cycles instead of one; and (3) the polypetalous condition of the corolla. Because of their significance, each of these three is worthy of some discussion; and all may be brought to bear finally upon the taxonomic position of the family.

Bisporangiate stamen

Three classes of facts lead to the conclusion that the bisporangiate stamen is in itself complete, and that the tetrasporangiate one is double. In the first place the tetrasporangiate stamen, as described for *Echinocystis*, has its origin in two primordia that are distinctly separate. Baillon (1) describes exactly the same state of affairs for *Bryonia*, as does also Payer (quoted by Baillon). In these instances at least the larger stamen is double in its origin. Union of its two components, whatever the method of it, occurs subsequently and is incidental rather than fundamental. It is true that Heimlich's (6) figures of *Cucumis* do not show two primordia for each tetrasporangiate stamen, but of this fact there are two possible explanations, neither of which is contradictory to the present conclusion. It is possible that Heimlich's figures do not represent young enough primordia at the right cross-section level, or, if they do, it is possible that meristematic activity between the paired primordia begins so early in this species that the two are never visibly distinct.

In the second place the staminodium habit in pistillate flowers strongly suggests the double nature of the tetrasporangiate stamen. The pistillate flower of *Fevillea*, according to Baillon, has five staminodia which correspond with five bisporangiate stamens in the staminate flower. *Bryonia* also has five staminodia in its pistillate flower, but only three staminate masses in the staminate flower. That the three masses are really the expression of five stamens is indicated, not only by the mere occurrence of five staminodia, but also by the fact that these five separate organs are arranged into three groups, corresponding with the three masses in the staminate flower. *Echinocystis* reveals a similar situation; two staminate masses (three stamens) in the one flower and three staminodia, of which two are paired, in the other. Since subsequent growth changes in the stamen representatives are less apt to occur in the pistillate than in the staminate flower where the stamens are functional, distribution evidence and individuality evidence come, in the former, to have considerable force. Some genera, it is true, have staminodia in one flower equal in number to the staminate masses in the other, even when two such masses are each tetrasporangiate. Such cases are worth investigating, for the small number of staminodia may have resulted from re-

duction in the androecium, from abortion of some of its members, or even from combined unit growth such as has been described between two functional stamens.

In the third place *Thladiantha*, as described by TISON (quoted by BAILLON), stands intermediate in position between the five-separate-stamen situation and the five-stamen-mass situation, and it clearly shows the homology between the two. *Thladiantha* has five bisporangiate stamens, all of which are separate, but four of them are arranged in two pairs whose members, although not in any sense united, stand close together. The fifth is isolated without a mate. Here then are five stamens in three groups; not very different from the situation in *Cucumis*.

NAUDIN (18) refuses to grant the bisporangiate stamen the status of a complete organ: (1) because there is no evidence of fusion on the filaments of tetrasporangiate stamens; (2) because, in perfect flowers, the stamen masses are equal in number to the carpels and in fixed relation of symmetry with them; and (3) because of a monstrosity seen in *Lagenaria*, where the bisporangiate stamen had grown another "half" which was green and leaflike, but whose undulating margin simulated the S-shaped sporangia of the normal organ. In answer to the first contention it may be repeated that the tetrasporangiate stamen is double, not because two separate members have fused together, but, as already shown in *Echinocystis* and as intimated by VAN TIEGHEM (24), because of the rise of a growing point between two primordia such as to involve both in the development of one single staminate mass. NAUDIN'S second contention cannot stand in the face of genera whose staminodia are isomerous with petals rather than with carpels, and the third one falls in the absence of an explanation for the monstrosity. Such a monstrosity may result from any one of a number of varied conditions, and it proves the half nature of the bisporangiate stamen no more than petalody proves the normal flower to be but half complete.

HEIMLICH'S agreement with NAUDIN seems to rest on (1) the absence of vascular evidence for any missing stamens or stamen parts; (2) the sporangium number which, in the small stamen, is just half the number that is common to most stamens; (3) the alternation of stamen masses and of staminodia with carpels; and (4) the equality

in number between stamen primordia and mature stamens. Of course, if all five stamens are present in the flower, as I believe them to be, one could hardly expect to find vascular evidences of any missing ones. But even if some were missing there would likely be no vascular traces left for their identification, for vascular differentiation follows a growing point rather than precedes it; if there is no growing point there is small chance of there being any bundles. The absence of telltale traces then cannot be interpreted as evidence that three is the necessary stamen number in *Cucumis* and two in *Echinocystis*, nor is it evidence that there were never five stamens in the former and three in the latter. While agreeing with HEIMLICH that the number of bundles in an organ cannot be regarded as proof of its homology, I cannot refrain from mentioning the strong suggestion which the double bundle has in each tetrasporangiate stamen of *Cucumis* as contrasted with the single bundle in the bisporangiate stamen. Similarly the number of sporangia per staminate mass cannot be given much weight in settling homologies, for it is well known that variation exists in this respect among angiosperms. From what may be regarded as a single sporangium in the stamen of *Lemna* there is progression up to large numbers in the branched stamens of such forms as *Ricinus* and *Callothamnus*, and two sporangia per stamen is the normal situation in the Asclepiadaceae. Alternation of staminodia or stamen masses with carpels can hardly be significant, in view of the bundle associations already mentioned in the pistillate flower of *Echinocystis*, and in view of the numerous cases in which carpels and staminodia are not equal in number. Finally, the matter of numerical equality between stamen primordia and mature stamens has already been disposed of earlier in this paper.

On the basis of evidence at hand, therefore, it is my conviction that the bisporangiate stamen in Cucurbitaceae is a complete stamen; in no developmental sense at all is it a half organ. On this basis it is probable that *Telfairia* has ten stamens produced in five pairs; that *Fevillea* has five separate stamens; that *Thladiantha*, also with five, has its stamens in three groups; that *Cucumis* has five stamens, four of which are united in two masses; and that *Cyclanthera* has five stamens all united in one mass. *Echinocystis*, on a trimerous plan, has three stamens, two of which are united in one mass.

PENTACYCLIC SITUATION

The ten stamens of *Telfairia* furnish indirect evidence of two stamen cycles in that genus, while the present investigation furnishes direct evidence of the same situation in *Echinocystis*. If two stamen cycles are at all common in the family, it is anything but surprising that VAN TIEGHEM (24) and EICHLER (4) should disagree as early as they did over the question of stamen distribution, the former seeing stamens opposite the petals and the latter seeing them opposite the sepals. Nor is it strange that BAILLON (1) should see the single stamens opposite the sepals and the double ones opposite the petals. Further investigation is needed to determine how common this matter is, and also to determine whether or not combined stamens are always members of different cycles, as they apparently are in *Echinocystis*.

POLYPETALY

The idea of polypetaly in the Cucurbitaceae is by no means new, particularly to those taxonomists whose acquaintance with the family is not limited to our common North American forms. NAUDIN (18) goes so far as to say that only exceptionally are the petals fused. BAILLON (1) says of the group in general that they are clearly polypetalous, and he speaks also of five free petals in *Bryonia*. In BAILEY'S *Manual* and again in BRITTON and BROWN'S *Flora* the polypetalous condition is described for a part of the family, but it seems not to have been realized that our sympetalous genera too may be fundamentally polypetalous, as described for *Echinocystis*. HEIMLICH does not describe the corolla situation in *Cucumis*, and although he does mention vascular bundles which occupy positions exactly between the corolla lobes, even these would not exclude the possibility of petal individuality, since essentially the same thing is seen over and over again in the intercarpellary bundles of compound ovaries.

That polypetaly is more widespread in the family than is superficially apparent there is no doubt. Here again more investigation is needed, since it is the tubular corolla of the Cucurbitaceae that has been responsible for the inclusion of this family with the Sympetalae in the ENGLER and PRANTL system.

Conclusion

WERNHAM (25) has summarized the morphological characters of the Cucurbitaceae which have led to the inclusion of the family in the Sympetalae, and has adversely criticized each one. He mentions particularly the matter of cohesion in the androecium, that of the multilocular ovary, and that of the tubular corolla. But it is the adherence of anthers by their edges, as in Compositae and Lobeliaceae, that marks the evolutionary tendency in the upper sympetalous families, and this character is fundamentally different from the unison growth of stamen filaments and stamen connectives in the Cucurbitaceae. Likewise the multilocular ovary of the Sympetalae, divided up as it is by the radial walls of the several involved carpels, differs fundamentally from the cucurbitaceous ovary, which is basically unilocular, but which becomes multilocular secondarily by the enormous ingrowth of its parietal placentae. And finally the sympetalous character itself of the Sympetalae is fundamentally different from that of many, at least, of the Cucurbitaceae.

When to the foregoing there are added the facts that the Cucurbitaceae, unlike other Sympetalae, have ovules with two integuments and a large nucellus, and that, unlike other high Sympetalae, its flowers are prevailingly monoecious or dioecious, there can hardly be much surprise occasioned by WERNHAM'S expression, "Cucurbitaceae with somewhat doubtful affinity." In BENTHAM and HOOKER'S system, as also in WARMING'S, this family was included with the Archichlamydeae in spite of its apparently united petals. And now, with doubt cast on the sympetalous character of its flowers, there is no reason left for retaining the family in the group of sympetalous "foreigners."

The family, or at least a part of it, must also be degraded from its supposed tetracyclic position to the humbler pentacyclic one. There is evidence of variation in this character, but it would be expected anyway since the family is clearly a border-line one.

Summary

1. The order of appearance of organs in floral ontogeny is sepal, petal, stamen. Carpels are not represented.

2. The growth of floral organs is accomplished by means of both cell division and cell enlargement. Neither of these processes is restricted to a definite meristem or cambium.

3. Sepals are completely separate and therefore there is no calyx cup. Each sepal is traversed by a single bundle which enters from the receptacle.

4. While the petals form a loose corolla tube, each has its own epidermis which completely and distinctly marks it off from its adherent neighbors, and which clearly establishes its individuality.

5. Each petal is traversed by five bundles, all of which enter from the receptacle. All but the median bundle are derivatives of sepal bundles.

6. The completeness of the bisporangiate stamen is believed established by the fact that the tetrasporangiate stamen has its origin in two separate primordia; by the fact that staminodia in the pistillate flower, although disposed like stamen primordia, commonly develop without combining in pairs; and by the fact that *Telfairia*, with its stamens in an intermediate position between two extremes, clearly shows the homology between a tetrasporangiate stamen and two bisporangiate stamens.

7. Vascular associations between staminodia and perianth members in the pistillate flower indicate that the three stamens are members of two stamen cycles; two are opposite petals and one is opposite a sepal.

8. Each division of the androecium, whether bisporangiate or tetrasporangiate, is traversed by a single bundle. This bundle gives off one branch in the former and two in the latter. It is connected directly with the vascular anastomosis in the base of the receptacle rather than with bundles which supply the perianth.

9. With polypetaly known in the family there remains no longer a reason for including Cucurbitaceae in the Sympetalae. And with the pentacyclic condition known in some members, the family comes to take on more aspects of a highly variable, border-line group.

It is a pleasure to acknowledge here the stimulating suggestions and criticisms which were so freely given by Professors C. J. CHAMBERLAIN and E. J. KRAUS, of the University of Chicago, during the

progress of this research. It is also a pleasure to acknowledge the contribution made by Miss DOROTHY B. SMITH in securing original papers to which reference has been made and which were not accessible to the writer.

SOUTH DAKOTA STATE COLLEGE
BROOKINGS, S. D.

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EXPLANATION OF PLATES XIII–XVI

PLATE XIII

FIG. 1.—Diagram of androecium: *A*, habit showing face view of tetrasporangiate stamen; *B*, transverse section showing each pair of sporangia cut three times; *s*, bisporangiate stamen; *s'*, tetrasporangiate stamen.

FIG. 2.—Longitudinal section of flower primordium; ×200.

FIG. 3.—Longitudinal section of young flower with concave receptacle; ×200.

FIG. 4.—Longitudinal section of young flower with primordia of organs: *p*, petal; *s*, sepal; ×200.

FIG. 5.—Longitudinal section of young flower with stamen primordia appearing on floor of receptacle; ×200.

FIG. 6.—Longitudinal section of young flower with stamen primordia distinct and receptacle beginning to elongate; ×200.

FIG. 7.—Longitudinal section of young flower with petals and sepals carried above stamens on receptacle: *p*, petal; *r*, receptacle; *s*, sepal; *st*, stamen; ×200.

FIG. 8.—Transverse section of young flower at stage in fig. 6, showing three stamen primordia with two close together; ×200.

FIG. 9.—Transverse section of young flower at stage in fig. 7, and taken at petal level; ×200.

FIG. 10.—Longitudinal section of slightly older flower, showing lobed character of right-hand stamen; ×200.

PLATE XIV

FIG. 11.—Longitudinal section of flower showing early lobing of stamens: *p*, petal; *r*, receptacle; *s*, sepal; *st*, stamen; ×80.

FIG. 12.—Transverse section of flower at same stage as that in fig. 11, showing double nature of large stamen after establishment of growing point between two original primordia; ×80.

FIG. 13.—Longitudinal section of older flower with sporangia organized; note two stamen masses clearly separate; ×80.

FIG. 14.—Transverse section of flower at same stage as that in fig. 13: *m*, microsporangium; *s*, tetrasporangiate stamen; *s'*, bisporangiate stamen; *v*, vascular bundles; ×80.

FIG. 15.—Longitudinal section of older flower showing two stamen masses grown together above stalklike bases; ×52.

FIG. 16.—Cells from receptacle of fig. 6; ×410.

FIG. 17.—Cells from receptacle of fig. 7; ×410.

FIG. 18.—Cells from receptacle of fig. 15; ×410.

FIG. 19.—Cells from receptacle of mature flower; ×410.

FIG. 20.—Transverse section of receptacle cup of mature flower; ×275.

FIG. 21.—Section through stoma of mature receptacle; ×410.

FIG. 22.—Longitudinal section of glandular hair from upper and inner surface of receptacle; ×275.

FIG. 23.—Glandular hairs from lower and inner surface of receptacle: *A*, longitudinal section; *B*, *C*, transverse sections through terminal gland; ×275.

PLATE XV

FIG. 24.—Longitudinal section of hair from outer receptacle surface; ×275.

FIG. 25.—Cells from sepal of fig. 6; ×410.

FIG. 26.—Cells from sepal of fig. 7; ×410.

FIG. 27.—Cells from sepal of fig. 15; ×410.

FIG. 28.—Cells from petal of fig. 6; ×410.

FIG. 29.—Cells from petal of fig. 7; ×410.

FIG. 30.—Cells from petal of fig. 15; ×410.

FIG. 31.—Cells from petal of mature flower; ×410.

FIG. 32.—Transverse section through "joint" of corolla tube just above receptacle; dotted line marks boundary between petals; ×410.

FIG. 33.—Same as fig. 32, but about one-third up length of corolla tube; dotted line marks boundary between petals; ×410.

FIG. 34.—Same as fig. 32, but about half up length of corolla tube; here it is impossible to trace the two epidermal layers through "joint"; ×410.

PLATE XVI

FIG. 35.—Same as fig. 32, but up at level where petals are beginning to diverge from one another; intercellular spaces appear between the two epidermal layers; ×352.

FIG. 36.—Longitudinal section of young petal with epidermal hairs; ×352.

FIG. 37.—Transverse section through boundary region between bisporangiate and tetrasporangiate stamens: *c*, schizo-lysigenous cavities; *e*, epidermal layers of two stamens; ×235.

FIG. 38.—Transverse section of young anther with primary sporogenous cell that grows into spore mother cell: *e*, stamen epidermis; *p*, primary parietal layer; ×570.

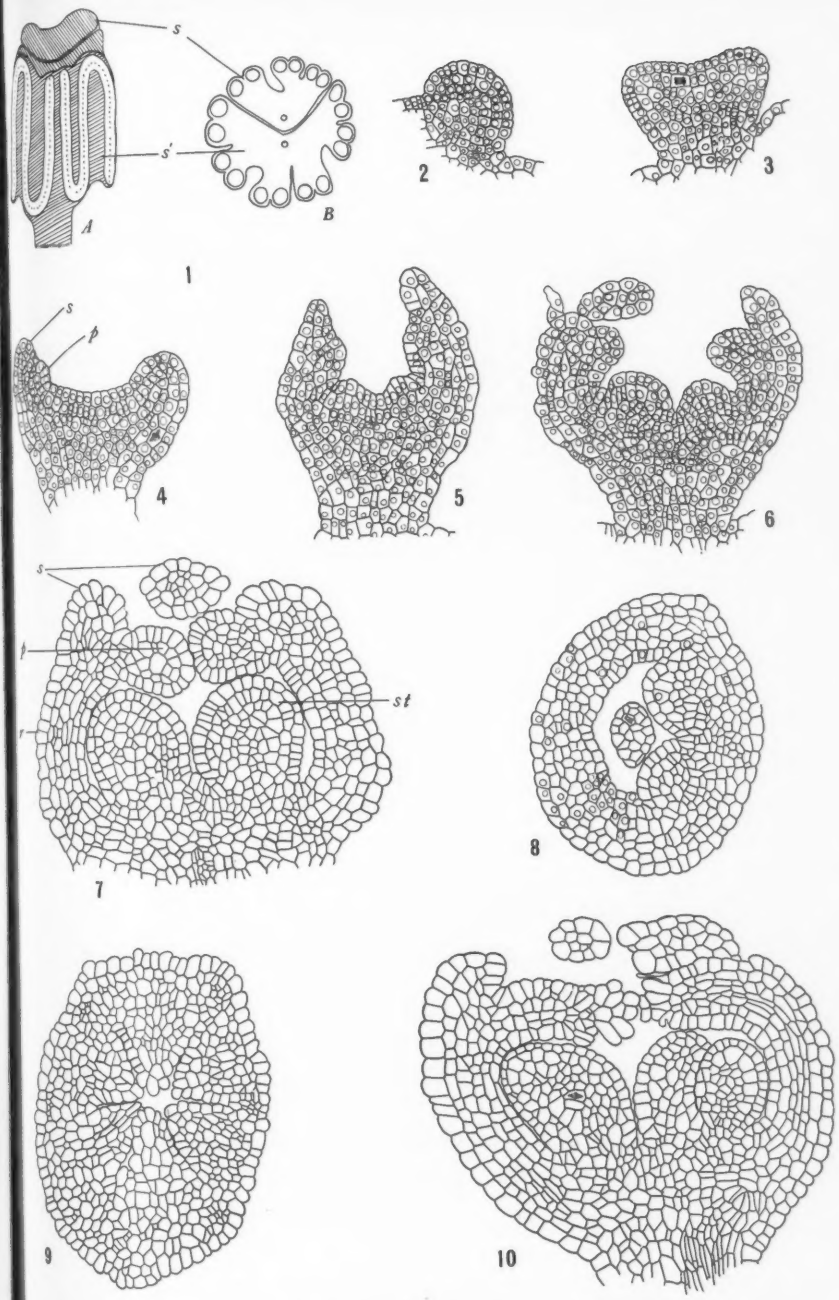
FIG. 39.—Transverse section of young anther whose sporangium wall is developing from primary parietal cells: *e*, stamen epidermis; *p*, parietal cells; ×570.

FIG. 40.—Transverse section of anther whose sporangium has developed three-layered wall: *t*, tapetum; ×570.

FIG. 41.—Diagram of round receptacle showing distribution of vascular bundles extending from basal, anastomosing tangle of short strands out to periphery where they will supply petals and sepals; not all twelve bundles connect directly with basal vascular mass.

FIG. 42.—Diagram of receptacle, petals, and sepals showing bundle distribution: *a*, anastomosing bundles at receptacle base; *p*, petal; *r*, receptacle; *s*, sepal.

FIG. 43.—Diagram of androecium showing bundle distribution: *b*, bisporangiate stamen; *t'*, tetrasporangiate stamen.



MILLER on ECHINOCYSTIS

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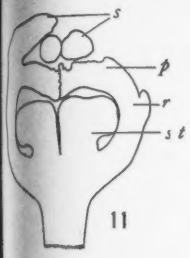

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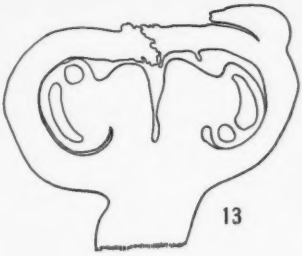

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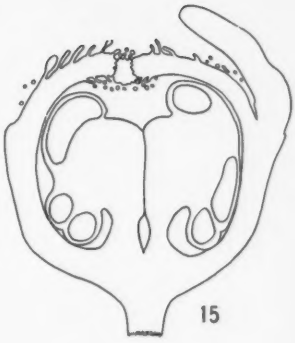

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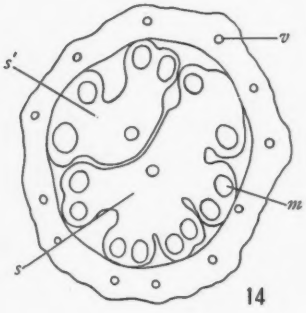

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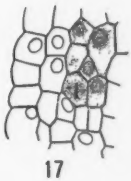

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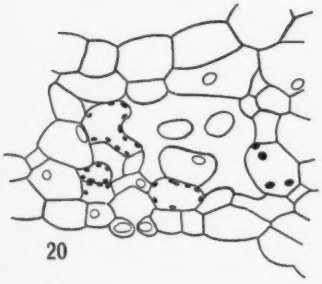

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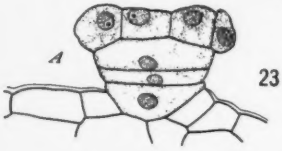

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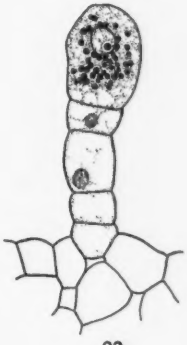

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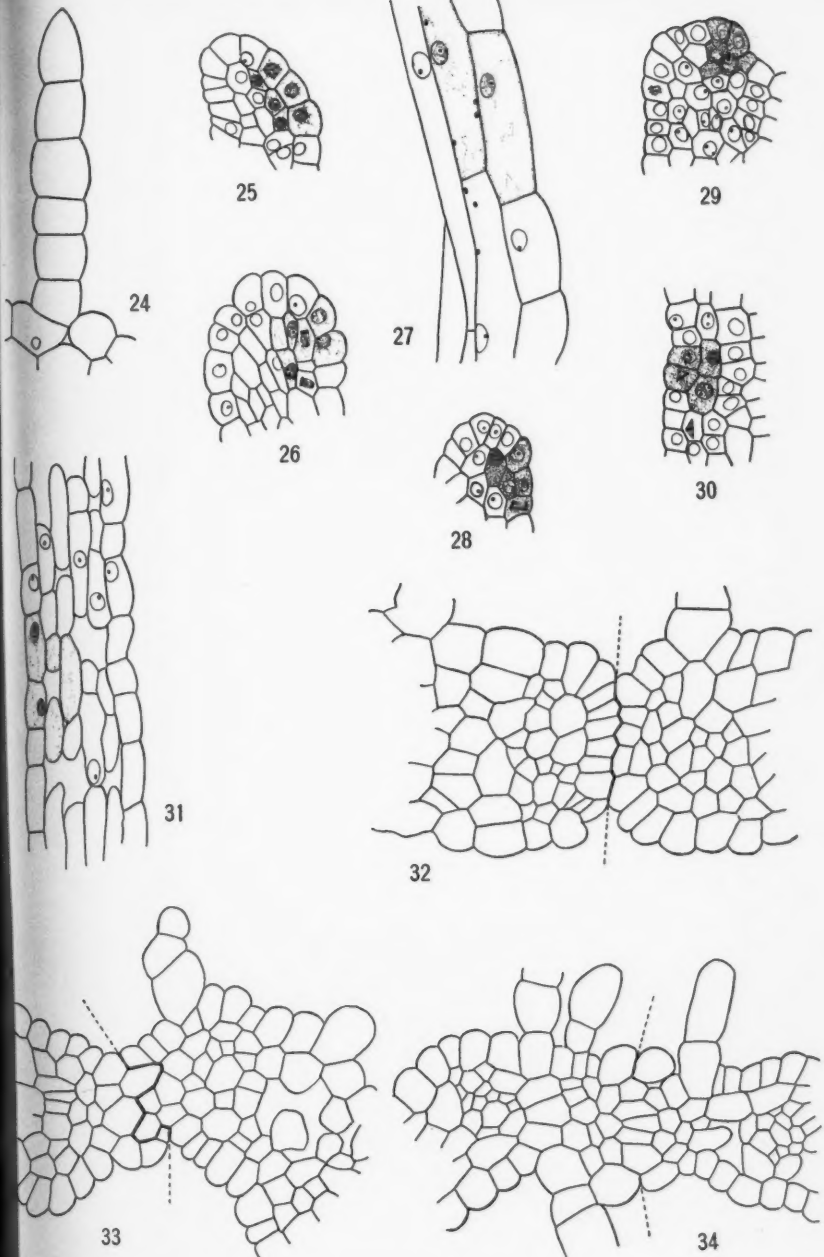

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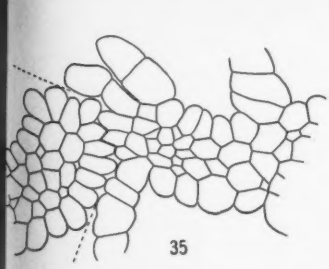

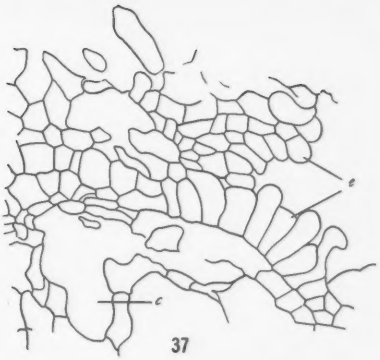

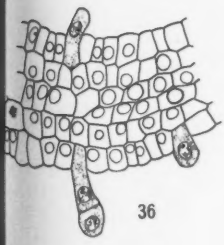

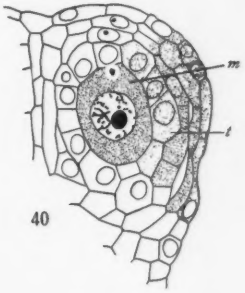

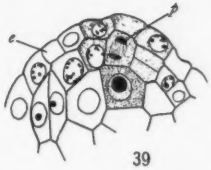

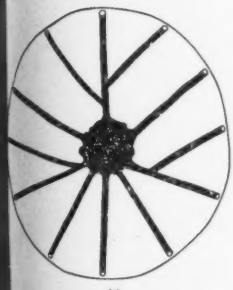

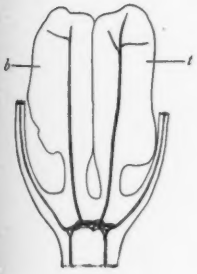

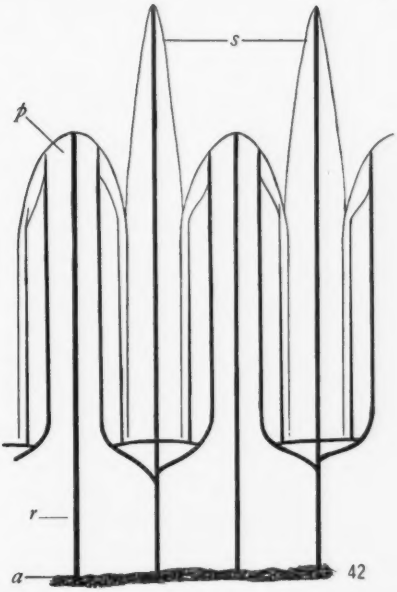

MILLER on ECHINOCYSTIS

NEW OR OTHERWISE NOTEWORTHY COMPOSITAE. III

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 397

EARL EDWARD SHERFF

(WITH PLATES XVII–XXI)

BIDENS PILOSA var. BIMUCRONATA f. ODORATA (Cav.) Sherff, BOT. GAZ. **81**:41. 1926; *Cosmos tenellus* H. B. K. Nov. Gen. et Sp. 4:188 (240). 1820; *Bidens inermis* Wats. Proc. Amer. Acad. **23**: 278. 1888. (pl. XVII)

To the list of synonyms for the forma *odorata* may be added also *Bidens inermis* Wats., a name founded upon *C. G. Pringle* 1291, in thin soil on rocky ledges, Arroyo Aucho, in the Sierra Madre, State of Chihuahua, Mexico, October 14, 1887 (type, Herb. Gray: cotypes, Herb. Berl.; Herb. Boiss.; Herb. Univ. Calif.; Herb. Field Mus.; Herb. Kew; Herb. Phila., etc.). *Pringle* 1638, 5999, and 7904 all are identical as to fruiting capitula with the type material of *B. inermis*. They all have the achenes especially slender and long-attenuate, as well as exaristate. The forma *odorata* is known, however, frequently to produce achenes wholly or in part of this very type, thus leaving no grounds for the retention of WATSON'S *B. inermis* as a species.[1] It may be remarked in passing that O. E. SCHULZ, in 1911, studied some of the *B. inermis* material (e.g., *Pringle* 1638) in the Berlin Herbarium and labeled it *B. caucalidea* DC., which in turn, as pointed out in my former article, is merely another synonym for f. *odorata*.

The type of *Cosmos tenellus* H. B. K. was collected between the City of Mexico and Huahuatoca (variously spelled Huehuetoca, Gueguetoque, etc.) in Mexico. A search by me in 1914 and again in 1924, among the type sheets of the HUMBOLDT, BONPLAND, and

[1] The singular aspect of the fruiting heads upon typical "*B. inermis*" might be taken as justifying a *subformal* rank under f. *odorata*, but the already lengthy name *B. pilosa* var. *bimucronata* f. *odorata* would seem to suggest restraint in this matter. Furthermore, some material (e.g., *Dr. Edward Palmer* 673, Herb. Kew) has fruiting capitula precisely like those in the type collection of *B. inermis* but merely 3-5-partite leaves as in *B. pilosa* var. *bimucronata* proper, thus displaying an overlapping of characters.

KUNTH set at Paris, failed to reveal a specimen with the name *Cosmos tenellus;* nor was there authentic material at Berlin. The description by KUNTH shows that the type was without achenes. The bipinnatipartite foliage and the probably rosaceous color of the ligules evidently had led him to place the plant in *Cosmos*. A survey of *Cosmos* and *Bidens* at the present time indicates that KUNTH's extended description fits none other than f. *odorata* of *Bidens pilosa* var. *bimucronata* (Turcz.) O. E. Schz. This forma, as well as its supravening variety *bimucronata* (with which it frequently intergrades), is very abundant throughout the region surrounding the type locality of *Cosmos tenellus*. Moreover, the general aspect is ofttimes deceptively like that of *Cosmos*, a fact easily explaining KUNTH's reference of his type to that genus.[2]

BIDENS ANGUSTISSIMA H. B. K. Nov. Gen. et Sp. **4**:183 (233). 1820; *B. angustifolia* H. B. K. *loc. cit.* **7**:359 (456). 1825.—Occasionally references to this species are found in herbaria and libraries but with the spelling *angustifolia*. KUNTH'S original description was under the name *angustissima*, a name that, from considerations of priority, must remain the valid one. The type was listed as growing "locis subfrigidis, prope Los Joares et Santa Rosa de la Sierra, alt. 1300 hex. . . . ." A search through the later volumes of KUNTH'S work shows, in the 7th volume (*loc. cit.*) the name *B. angustifolia*. That the same species was meant is shown, however, by the context, "prope Los Joares et S. Rosa. (1300 h.)."

BIDENS CORIACEA (O. Hoffm.) Sherff, BOT. GAZ. **81**:52. 1926; *Coreopsis coriacea* O. Hoffm., Engler Pflanzenw. Ost-Afr. 414. 1899; *C. ruwenzoriensis* S. L. Moore, Jour. Linn. Soc. **35**:345. 1902; *Bidens ruwenzoriensis* (S. L. Moore) Sherff, BOT. GAZ. **59**:309. 1915. (pl. XVIII)—In a former article (BOT. GAZ. **81**:52. 1926) I pointed out the similarity between the types of *B. coriacea* and *B. ruwenzoriensis*, but was reluctant to merge the two into one species because of the lack of intermediate material. Since then I have studied specimens collected by *Sir Evan James*, Libu, Nandi Country, Uganda, British East Africa (Kew). These have the achenes *biaristate* as in the type

[2] It may be noted that several specimens collected by *Brother G. Arsène* in the States of Puebla and Michoacan (U. S. Nat. Herb.) have been distributed by *Arsène* under the name *Cosmos tenellus* H.B.K. and that these are the forma *odorata* of *B. pilosa* var. *bimucronata*.

of *B. ruwenzoriensis*, but the outermost bracts not *much longer* than the others. With this connecting form at hand, there appears no justification for attempting the further retention of *B. ruwenzoriensis* as a separate species.

BIDENS TRIPLINERVIA **nematoidea** var. nov.—A specie foliis numerosissimis et pinnatis vel bipinnatis, segmentis capillaribus plerumque tantum circ. 0.3–0.6 mm. latis differt.

H. L. Viereck 5, Cerro Quemado, Santa Marta, Colombia, Dec. 17, 1922 (type in U. S. Nat. Herb.); *Herbert H. Smith* 1980, rare on open lands, alt. 6500–7500 ft., San Lorenzo Ridge, Santa Marta, Colombia, Jan. 26, 1899 (Herb. N. Y. Bot. Gard.).

In 1913 the *Smith* plant from Santa Marta was noted by me as a singular foliage form and photographs (my photograph no. 341) were distributed to several herbaria. Recently I have been sent the *Viereck* plant, collected in the same locality and revealing similar characters. On both specimens the segments of the very numerous and pinnately or more often bipinnately parted leaves are strikingly threadlike. Otherwise the plants are like those of the very common var. *macrantha* (Wedd.) Sherff.[3]

BIDENS BIGELOVII **pueblensis** var. nov.—Folia principalia valde membranacea, tenuiter petiolata petiolis usque ad 3 cm. longis, petiolo adjecto 5–7 cm. longa, circumambitu triangulato-ovata, pinnata vel bipinnatisecta, segmentis primariis ovatis saepe 2–3 cm. longis et 1.2–1.6 cm. latis. Flores ligulati circ. 5, subflavi, tantum circ. 6–7 mm. longi. Achaenia inferne glabrata superne plus minusve erecto-setosa, apice biaristata aristis stramineis 1.5–3 mm. longis, retrorsum hamosis, dimorpha, exteriora clavata, badia vel rubro-straminea, corpore tantum circ. 4.5–5.5 mm. longa, interiora atra corpore usque ad 11 mm. longa et superne attenuata.

G. Arsène 7211, vicinity of Puebla, State of Puebla, Mexico, October, 1908 (type, U. S. Nat. Herb.); *idem* 5870, alt. 1950 m., Loma Santa Maria, vicinity of Morelia, State of Michoacan, Mexico, Sept. 4, 1910 (U. S. Nat. Herb.).

The foliage of *Arsène's* plants suggests strongly that of *Bidens duranginensis* Sherff, but the dimorphic achenes reveal the affinity

[3] The capitula are 5-rayed; the mature achaenia (6–8 mm. long) are exaristate (as sometimes happens in the var. *macrantha* too) and are somewhat surpassed by the paleae. *Smith* reported the plants as growing erect and up to 18 inches high.

with *B. bigelovii* Gray, a species which, in its typical form, is confined to a more northern range.

BIDENS TRIPARTITA **cernuaefolia** var. nov.—Folia indivisa vel summa subtripartita, breviter petiolata petiolis alatis circ. 1 cm. longis, petiolo adjecto 7–9 cm. longa, oblongo-lanceolata, membranacea, glabra, leviter serrulata, apice acuta sed parce acuminata, base sensim rotundata. Achaenia cuneato-oblanceolata, plana, purpurascenti-atra, faciebus glabra striataque, marginibus retrorsum hamosa, corpore 7–8 mm. longa et 1.5–2 mm. lata, apice biaristata aristis retrorsum hamosis 3–4 mm. longis.

R. C. Ching 4672, rare along open ditch of water, alt. 400 feet, Pei Chen, Province of Anhwei, China, Sept. 15, 1925 (type in Herb. Univ. Calif.).

Among the probably more than two thousand sheets of *Bidens tripartita* material studied by me, all specimens heretofore examined have proved referable either to the species proper or to one of three varieties, viz.: var. *hirta* (Jord.) Sherff, var. *orientalis* (Velen. *ex* Bornm.) Sherff, and var. *repens* (Don) Sherff. This plant, from the little known Province of Anhwei, appears to represent a variety quite distinct from any of the others. The leaves bear a strong resemblance to those found in some forms of *Bidens cernua* L. and of *B. laevis* (L.) B. S. P.

Bidens schizoglossa sp. nov.—Frutex ±8 dm. altus, caule glabro, ramis subquadrangulatis subpurpurascentibus, glabris, internodiis quam foliis multo brevioribus. Folia tripartita, petiolata petiolis tenuibus eciliatis 1.5–3.5 cm. longis, petiolo adjecto 7–13 cm. longa, glabra, foliolis membranaceis, acriter dentibus terminaliter cuspidatis serratis vel (foliis summis) etiam integris, acuminatis, lateralibus lanceolatis et basaliter valde obliquis, sessilibus vel parce petiolulatis, 4–6 cm. longis et 1.5–2.5 cm. latis, terminali saepius ovato-lanceolato, oculis petiolulato, lamina 5–7 cm. longo et usque ad 4 cm. lato. Capitula numerosissima, corymboideo-paniculata pedicellis ultimis pubescentibus saepius tantum 2–4 mm. longis, radiata, pansa ad anthesin circ. 7–10 mm. lata et 3.5–5.5 mm. alta. Involucri pubescentis bracteae exteriores 4 vel 5, lineares, apice saepius subobtusae, tantum circ. 1.5 mm. longae, interiores lanceolatae circ. 2.5–3 mm. longae. Flores ligulati 5 vel etiam 6, flavidi, ligula obovati vel

saepe obdeltoidei, 3–4 mm. longi, apice moderate vel saepissime profunde perspicueque scissi, lobis 2 vel 3, terminaliter acutis subacutisve. Achaenia submatura linearia, plana, brunneo-atra, faciebus glabra et leviter paucistriata, marginibus parce subalata et inferne saepe setis elongatis erectis adnatis 1-3-setosis, sub vel fere ad apicem biaristata aristis calvis vel 1–3 hamis retrorsum hamosis, apice ipso plerumque erecto-setulosa, corpore ipso 7–8 mm. longa et circ. 1.2 mm. lata, quam paleis paulo breviora. (pl. XIX)

W. A. and *C. B. Setchell*, near Huehue, Island of Hawaii, Hawaiian Islands, June 24, 1924 (type, Herb. Univ. Calif., no. 247073).

A species closely allied with *Bidens micrantha* Gaud. and its varieties. It differs from that species and its varieties in outline or dimensions of the leaves, in having smaller and much more numerous capitula (and these upon shorter pedicels), in having the ligules often deeply and conspicuously cleft at the apex (whence "*schizoglossa*") and probably also in characters of the mature achenes, which last are lacking on the type. *B. ctenophylla* Sherff, the type of which (*Degener* and *Wiebke* 2128) came from the same locality, differs from *B. schizoglossa* in having different foliage, fewer and larger capitula, larger, differently shaped and entire or apically obscure-denticulate ligules, etc., although both species are similar in having minute, pubescent pedicels.

Bidens obtusiloba sp. nov.—Frutex glaber, ±6 dm. altus, ramosus ramis quadrangulatis, internodiis inferioribus quam foliis plerumque multo brevioribus. Folia tenuiter petiolata petiolis usque ad 3.5 cm. longis, petiolo adjecto usque ad 8 cm. longa, bipinnati-(plerumque biternati-) secta segmentis primariis circumambitu oblongo-ovatis vel saepe deltoideis, membranaceis, obsolete ciliatis, 1.5–3.5 cm. longis et paulo angustioribus, lateralibus breviter petiolulatis, omnibus in lobos vel dentes obtusos ac apice minute cuspidatos rursus dissectis. Capitula non numerosa, plus minusve corymbosa, radiata, pansa ad anthesin 1–1.5 cm. lata et 5–7 mm. alta, tenuiter pedicellatis pedicellis usque ad 1.8 cm. longis. Involucri nunc glabri nunc basaliter pubescentis bracteae exteriores 4 vel 5, lineares, apice subacutae, circ. 2 mm. longae, quam interiores lanceolatae dimidio breviores. Flores ligulati circ. 4 vel 5, flavidi, ±5 mm. longi, an-

guste obovati, apice 2- vel 3-dentati. Achaenia linearia, atro-brunnea, valde obcompressa, singula facie circ. 8-striata, duabus faciebus non nisi summam versus setosa, marginibus erecto-setosa, apice exaristata sed erecto-hispida, corpore 6–7.5 mm. longa et circ. 1 mm. lata. (pl. XX)

D. Le Roy Topping 2941, Niu Ridge, Island of Oahu, Hawaiian Islands, Nov. 30, 1924 (type, Herb. Univ. Calif. no. 305183).

Nearest apparently to *Bidens pulchella* (Less.) Schz. Bip., a species known only from its diminutive type specimen collected a century ago by *Chamisso* on the same island (*cf.* BOT. GAZ. 85:25, pl. II, figs. *j-p.* 1928). The type of *B. pulchella* (Herb. Berl.) is a complete plant but only about 12 cm. tall, has the several principal leaves tripinnatisect with narrower segments than in *B. obtusiloba*, and has achenes distinctly clavate and only about 3 mm. long. Its aspect is that of an annual but this doubtless is due to the fact that the plant had been gathered in its first year, whereas the type of *B. obtusiloba* is a branch coming from a well developed shrub. A study of *B. obtusiloba* in its juvenile stages is much to be desired.

Bidens amphicarpa sp. nov.—Herba annua, gracilis, ramosa, 1–6 dm. alta, caule quadrangulato ac saepe purpurascenti, nunc pubescenti nunc glabrato. Folia tenuiter petiolata petiolis usque ad 2 cm. longis, petiolo adjecto usque ad 5 cm. longa, pinnatim 3–5-partita, foliolis valde membranaceis, glabratis vel sparsim adpresso-hispidis, ciliatis et plerumque minutissime atromarginatis, unico latere grosse 1–4-serratis dentibus acerrime apiculatis, lateralibus ovatis, terminali lanceolato vel fere lineari. Capitula in pedunculis tenuissimis usque ad 1 dm. longis ramos terminantibus disposita, radiata, pansa ad anthesin tantum circ. 6–7 mm. alta et 8–10 mm. lata. Involucri bracteae exteriores 5–8, tenuiter lineares, basaliter tergo margineque superne tantum marginaliter hispidae, apice acerrime mucronatae, demum circ. 4 mm. longae, interioribus lanceolatis atrobrunneis vel atropurpurascentibus sed marginaliter albido-diaphanis apicaliter ciliatis plerumque subaequalia. Flores ligulati circ. 5, ligula oblanceolati, 3–5 striis percursi, subflavidi vel subrosacei, apice saepe 2-(3-)dentati, 5–6 mm. longi. Achaenia exteriora subplana, unica facie circ. 4-sulcata, infra parce supra valde erecto-setosa, badia vel rubro-straminea, corpore 5–8 mm. longa, interiora

tereti-quadrangulata, omnino 8-sulcata, maximam partem atra sed apicaliter brunneo-straminea, erecto-setosa, elongato-linearia et supra saepe cervicem formantia, corpore demum 9–15 mm. longa; omnia recta vel subrecta 2–3-aristata aristis tenuibus retrorsum hamosis, 2.5–4 mm. longis, demum saepe caducis.

T. S. Brandegee, Sierra de Laguna, Lower California, Jan. 23, 1899 (type in Herb. Univ. Calif., no. 134269).

Differs from glabrous or subglabrous forms of the similarly amphicarpous *Bidens anthriscoides* DC. in the dissection and outline of the leaflets, in its proportionately much longer external involucral bracts,[4] and in its straighter, less hispid, and never tuberculate achenes. The general aspect is somewhat like that in stunted, slender forms of *B. pilosa* var. *bimucronata* f. *odorata* (Cav.) Sherff, but the very narrow external bracts, the reddish outer achenes, the dimensions of the flowering and fruiting heads (the latter with fewer achenes) all distinguish it from that form.

BIDENS SUBALTERNANS **simulans** var. nov.—A specie foliis plus dissectis, segmentis plerumque plus minusve linearibus, differt.

P. Dusén, in cultivated places, Tlapcrussú, State of Paraná, Brazil, Feb. 29, 1912 (U. S. Nat. Herb.); *idem* 9432, in grassy, shrubby places, State of Paraná, Mar. 25, 1910 (Herb. Gray; Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.); *Dr. Wilhelm Herter* 787, alt. 30 m., clay soil, along roads, Toledo, Department of Canelones, Uruguay, May, 1927 (Herb. Gray; Herb. U. S. Nat.); *P. Jörgensen* 1283, Department of Andalgalá, Province of Catamarca, Argentina, Oct. 11, 1916 (Herb. Gray; Herb. U. S. Nat.); *idem* 1785, growing erect, 1 m. high, *eodem loco*, Oct. 1, 1917 (type, U. S. Nat. Herb.: cotype, Herb. Gray); *Dr. Otto Kuntze*, Province of Cordoba, Argentina, December, 1894 (Herb. N. Y. Bot. Gard.).

At times, *Bidens subalternans* DC. is found with the leaves delicately 2–3-pinnatisect and the segments linear, even narrowly so. Heretofore, when making herbarium determinations, I have included such forms under the species proper without distinction. In some cases, they suggest *B. bipinnata* L. so strongly as to create confusion.[5] In other cases, they display a slight approach to *B. exigua* Sherff.

[4] In *B. anthriscoides* DC. the outer bracts are only about one-half or three-fifths the length of the inner ones, notwithstanding DECANDOLLE'S original description ("involucri squamis . . . . inter se subaequalibus;" Prodr. 5:601. 1836).

[5] *B. platensis* Mang., described as a hybrid (An. Mus. Nac. Buenos Aires 24:230. 1913) between *B. bipinnata* L. (pistillate) and *B. pilosa* L. (staminate), should be compared with this variety.

BIDENS SUBALTERNANS **unipinnata** var. nov.—A specie foliis pinnatim 3–5-partitis foliolis lanceolatis vel ovato-lanceolatis differt.

Dr. Emil Hassler 11558, in the region of Lake Ypacaray, central Paraguay, February, 1913 (type in Herb. Gray); *L. R. Parodi* 7787, adventive in cultivations, Lanugasta, Chilecito, Province of La Rioja, Argentina, Jan. 31, 1927 (Herb. Gray).

Occasionally a South American specimen of *Bidens* is found with the general aspect of *B. pilosa* L., yet with the fruiting heads as in the closely related *B. subalternans* DC. (*Hassler* 11558 and *Parodi* 7787 are examples). A close inspection shows that the only difference from typical *B. subalternans* is in the once-pinnate (instead of twice-pinnate) leaves.

Bidens glabrata (Gray) comb. nov.; *B. lantanoides* var.? *glabrata* Gray, Proc. Amer. Acad. **5**:128. 1861.—The type material of *Bidens lantanoides* Gray came from the Island of Eimeo (known also as Morea), of the Society Islands. It was collected by the *United States Southern Pacific Exploring Expedition* under *Captain Wilkes*. In a former article (BOT. GAZ. **85**:pl. V. 1928) I have illustrated it.

On the same expedition two small branches of another shrub were collected at the Island of Tahiti of the same island group. These are preserved in the United States National Herbarium. They are the types of GRAY'S doubtfully advanced "var.? *glabrata*." While very fragmentary as to capitula, they nevertheless are complete enough to reveal important specific differences from specimens of *B. lantanoides* proper and indeed from any other species of *Bidens*. As they appear fully worthy to rank as separate species and since GRAY'S description was much too brief, they were recently restudied by me and the following amplified description obtained:

Frutex, ramis subtetragonis, hinc inde hispidulis, minute striatis. Folia breviter ac late petiolata petiolis basaliter ciliatis circ. 1 cm. longis, petiolo adjecto 7–10 cm. longa et 2–3 cm. lata, oblonga, basaliter sensim apicaliter subabrupte attenuata, apice breviter acuminata, lamina glabra, crassiuscula, acriter serrata unico lateri 11–19 dentibus. Capitula corymboideo-paniculata, pedunculata pedunculis validis usque ad 8 cm. longis, verisimiliter radiata, involucris demum basaliter ± 7 mm. latis. Involucri bracteae exteriores circ. 7, late lineares, apice subobtusae, tergo non pubescentes nisi

basim versus, circ. 4 mm. longae; interiores oblongo-lanceolatae, 6–7 mm. longae. Achaenia submatura brunnea, matura atra, valde obcompressa, linearia, nunc inferne nunc superne attenuata, glabra, exalata, unica facie circ. 4-sulcata, corpore 4–5 mm. longa et circ. 0.8 mm. lata, apice biaristata aristis tenuibus retrorsum hamosis usque ad 2.2 mm. longis.

BIDENS TENERA **tetracera** var. nov.—Capitula discoidea ad anthesin 4–5 mm. alta et pariter lata. Involucri bracteae exteriores circ. 7 vel 8, lineares, tergo fere glabrae, margine ciliatae, sub apice interdum subdilatatae, apice ipso subacutae, circ. 3 mm. longae; interiores lanceolatae, 4–5 mm. longae. Achaenia linearia 15–30 in unico capitulo, fere usque ad apicem atra, apice ipso straminea, corpore exalata tetragona ac 1–1.5 cm. longa, circ. 0.5–0.65 mm. crassa, superne sensim attenuata, glabra, unica facie (4 facierum) 2-sulcata, apice quadriaristata aristis tenuibus, 2–3 mm. longis, retrorsum hamis elongatis tenuibus albidis hamosis.

Dr. Otto Buchtien 4182, at altitude of 1300 meters, Milluguaya, North Yungas, Bolivia, December, 1917 (type in U. S. Nat. Herb.); *H. Pittier* 10222, alt. 800–1000 m., around Caracas, Venezuela, Mar. 10, 1922 (Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.).

The type of *Bidens tenera* O. E. Schulz was collected in Costa Rica. At least twelve additional collections have been made, ranging southward and thence eastward into Colombia, Venezuela, French Guiana, and Brazil. At times, the species grows to a height of 5 or 6 decimeters, has tripartite leaves, produces as many as 20 achenes to a head, and then approaches the *Buchtien* plant from Bolivia. The latter, however, is still quite distinct in its more numerous achenes. These, moreover, are quadriaristate and upwardly attenuated, not triaristate and almost parallel-sided throughout as in the species' type. The general aspect is that of a thin-leaved form of *B. pilosa* L. as to foliage and of *B. bipinnata* L. as to fruiting heads.

Bidens biternata (Lour.) Merrill and Sherff, comb. nov.; *Coreopsis biternata* Lour. Fl. Cochinch. edit. I: 508. 1790; *ibid.* edit. II: 622. 1793; *Bidens chinensis* Willd. Sp. Pl. 3: 1719. 1804; *Actinea biternata* (Lour.) Spreng. Syst. 3: 474. 1826.—O. E. SCHULZ, in his special study of *Bidens chinensis* Willd. and related species (Engler Bot. Jahrb. 50 [Supplem.]: 178. 1914), appears to have overlooked the

Coreopsis biternata of LOUREIRO, published with a description in 1790, some fourteen years earlier than the date of WILLDENOW's work. In view of the many difficulties involved in the interpretation of some of Loureiro's species, I have refrained until recently from attempting final disposition of his names. At last, however, these difficulties have been overcome and a certain and conclusive treatment becomes possible.

LOUREIRO described two species of *Bidens*, namely *B. pilosa* L. and *B. bipinnata* L., and two of *Coreopsis*, namely *C. leucorrhiza* Lour. and *C. biternata* Lour. The first two may be passed over here, since they were admittedly not new species and since, moreover, the known occurrence of these two species in the region mentioned by LOUREIRO (Cochin China and China) tends to confirm the identity of the LOUREIRO plants. The third species, *Coreopsis leucorrhiza* Lour., has recently been referred by me (BOT. GAZ. **86**:443. 1928) to *Bidens pilosa* var. *minor* (Bl.) Sherff.

The fourth species, *C. biternata* Lour., was known to LOUREIRO under the Annamese dialectic name *Ca ap chioc*,[6] and he stated that it grew in fields near Canton, China. His description follows: "Differ. spec. Cor. foliis biternatis, ovato-lanceolatis, serratis: panicula diffusa: radio sexfloro. Habitus et notae. Caulis herbaceus, 3-pedalis, erectus, 4-gonus, 4-sulcatus, integre luteus: panicula sparsa, terminali. Radii corollae 6, neutrae. Pappus bicornis, ramosus. Receptaculum planisculum, nudum."

His character for the foliage, *biternatis*, shows at once that he was dealing with the same plant as WILLDENOW treated under the name *Bidens chinensis*. His "*pappus bicornis*" was doubtless merely one of

[6] LOUREIRO "lived at Hue for approximately thirty-five years, this town being the ancient capital of the kingdom of Cochin China, now a part of French Indo-China. I assume that the limits of the old kingdom of Cochin China were approximately the limits of the Province of Annam [Anam] to-day, in French Indo-China. On leaving Hue, Loureiro proceeded to Canton and spent two or three years there before proceeding to Lisbon. . . . . Loureiro tried to indicate in his native names as between Annamese (indicated by the letter "a") and Chinese names (really in Mandarin) by the letter "b" . . . . by China he means the general vicinity of Canton in Kwangtung Province. . . . . Generally speaking: I am of the opinion that most of his work was done on Cochin China specimens, chiefly for the reason that he resided so long in Cochin China and for a comparatively brief time in Canton; and the conditions in Canton at the time of his visit were such that it would have been impossible for him to visit any regions outside of the immediate vicinity of the city." . . . . DR. ELMER D. MERRILL *in litt.* April 22, 1929.

the numerous errors for which his descriptions were noted.[7] His "receptaculum . . . . nudum" probably was based upon capitula that had shed their achenes and chaff scales, for just previously, in his generic description of *Coreopsis*, he had said "recept. paleaceum."

In my monographic study of *Bidens*, I have found a considerable number of specimens from the general region traversed by LOUREIRO and which may be cited in confirmation of his description.[8] A few of these are:

Mr. Balansa 910, near Quang-yen (Kwangyen), Tongking, (French) Indo-China, August, 1885 (Herb. Par.); *R. P. Bodinier*, Hongkong, China (Herb. Par.); *Eberhart* 2568, Hoi-mit, Indo-China (Herb. Par.); *idem* 3338 *bis*, Thanmoi (Than Muoi), Province of Langson, Cochin China (Herb. Par.; *nom. indig.* Cay nu ao); *F. Evrard* 154, near Saigon, Cochin China, Oct. 21, 1920 (Herb. Par.); *idem* 225, Dalat, Indo-China (Herb. Par.); *Gaudichaud*, Macao, Province of Kwang-tung, China, 1836–1837 (Herb. Par.); *A. Germain* 109, Cochin China (Herb. Par.); *G. W. Groff*, around fruit trees, Sun Ooi, Lai Ngok Village, Canton Delta, Kwang-tung Province, China, Mar. 18, 1918 (Herb. Univ. Calif.; *nom. incolarum* Kam p'un ngan chan); *Hana* 298, Hongkong, China (Herb. Gray); *Dr. Harmand*, Iles de Poulo-Condor, Cochin China, 1875–1877 (Herb. Par.); *Dr. Aug. Henry* 8269, Isl. of Hainan, China, November, 1889 (Herb. Berl., 2 sheets); *H. Lecompte* and *A. Finet* 1252, Barka, Indo-China, November, 1911 (Herb. Par.); *iidem* 1733, Angkor Thom, Cambodia, Indo-China, Dec. 12, 1911 (Herb. Par.); *E. Lefèvre* 4, Cochin China, Sept. 25, 1864 (Herb. Par.); *F. A. McClure*, grassy field, Kingchow, Isl. of Hainan, China, Oct. 14, 1921 (Herb. Univ. Calif.); *idem*, roadside near Kingchow, Isl. of Hainan, Apr. 5, 1922 (Herb. Univ. Calif.); *Elmer D. Merrill*, bamboo thicket and border of dry thicket, Honam Isl., Canton, Kwang-tung Province, China, Oct. 13–Nov. 9, 1916 (Herb. Univ. Calif., 2 sheets: "this is the very common form at Canton. Honam Island is directly in front of the city, across the river. E.M."); *Mr. Petelot* 1211, roadsides, Hanoi Viuh, Cho Ganh, Tonking, Indo-China, November, 1922 (Herb. Par.) and November, 1923 (Herb. Univ. Calif., 2 sheets); *Dr. Talmy*, Cochin China, October, 1867 (Herb. Par.); *Dr. Thorel* 1270, Nareiager, Cochin China, 1862–1866 (Herb. Par., *quo pro Loureirone nominata*); *idem* (*similiter*) 1270, Bassac (Bassak), Cambodia, 1866–1868 (Herb. Par., 3 sheets).

Recently I was supplied by DR. ELMER D. MERRILL, Dean of The College of Agriculture of The University of California, with an

[7] Occasionally the pappus is *tricornis* and it may very well be that he had so spelled the word on sending it to the printer for the first edition. In any case, however, this error did not get corrected in the second edition.

[8] These mostly bear my herbarium determination, *Bidens chinensis* Willd. or (L.) Willd.

excerpt from page 553 of the as yet unpublished manuscript of his "A Commentary on LOUREIRO's Flora Cochinchinensis." It was interesting to find that MERRILL, too, had concluded *Coreopsis biternata* Lour. to be the same as *Bidens chinensis* Willd. Since then, he has very kindly assisted me with various desired data and now joins me in publishing the new combination which this conclusion makes necessary.

BIDENS BITERNATA var. **abyssinica** (Schz. Bip.) comb. nov.; *B. abyssinica* Schz. Bip. *in* Walpers Repert. **6**:167. 1846–1847; *B. chinensis* var. *abyssinica* (Schz. Bip.) O. E. Schz., Engler Bot. Jahrb. 50 (Supplem.):180. 1914.—This variety has been found, though rarely, as far northward as Arabia, British East India, Ceylon, the Provinces of Kiangsu and Shan-tung, China, and Corea. It is unknown, however, from French Indo-China or from Kwang-tung Province in southern China, in one or both of which places LOUREIRO obtained his type materials of *B. biternata* proper.

BIDENS SANDVICENSIS **typica** var. nov.; *B. sandvicensis* Less. *pro specie*, Linnaea **6**:508. 1831.

BIDENS SANDVICENSIS var. TYPICA **compositior** Deg. and Sherff, f. nov.—A var. *typica* foliis principalibus bipinnatisectis vel (saepe ternatim) bipinnatis differt. Achaenia biaristata aristis usque ad 1 mm. longis vel demum calvis.

Otto Degener and *Kazuto Nitta* 3411*a*, moderately dry, open slope at alt. 1500 feet, east rim of Manoa Valley, one mile mauka of the University, Isl. Oahu, Hawaiian Isls., Jan. 13, 1929 (type in Herb. Field Mus.); *iidem* 3412*a*, much windswept, sparingly wooded slope at alt. 2000 feet, east rim of Manoa Valley, one and one half mile mauka of University, Isl. of Oahu, Jan. 13, 1929 (Herb. Field Mus.).

Of twenty-seven collections of *B. sandvicensis* studied by me thus far, those by *Degener* and *Nitta*, nos. 3411 and 3412, were the first to be accompanied by a bipinnately leaved form. In each case only one specimen of this form had been found. The appearance of an entire plant is rendered strikingly unique by the dissection of the foliage. PROFESSOR DEGENER states that "apparently the more exposed to wind, the more parted are the leaves." In the case of the several dozen specimens sent me, however, the transition from the pinnate to the bipinnatisect or bipinnate type of leaves is very abrupt.

Greenmania sect. *Bidentis* nov.—Herbae Americae tropicae perennes, plerumque scandentes, caulibus saepe 5–10 m. longis; capitulis numerosis, normaliter radiatis; achaeniis elongatis, valde obcompressis vel omnino planis, lateribus parallelis et saepissime valde setosis, apice plerumque biaristatis aristis saepius longis nunc retrorsum hamosis nunc calvis.—**Type species,** *Bidens squarrosa* H. B. K.

The section includes a number of climbing species (*B. squarrosa* H. B. K., *B. rubifolia* H. B. K., *B. reptans* (L.) G. Don, *B. urophylla* Sherff, *B. speciosa* Gardn., etc.). DeCandolle (Prodr. **5**:596–599. 1836) employed the section *Psilocarpaea* for many very unlike species, merging the climbers with such species as *B. pilosa* L. and *B. andicola* H. B. K. Various considerations as to restricted geographic distribution, scandent stem-habit, clustered arrangement of capitula, shape of achenial body (flat, parallel-sided, usually hispid upon the margins, commonly biaristate, in appearance often suggesting a centipede), indicate the advisability of designating the climbing species under a distinct *sectional* name.[9]

BIDENS PILOSA var. RADIATA **indivisa** f. nov.—A varietate ipsa foliis indivisis ovato-lanceolatisque differt.

J. S. De La Cruz 1792, growing 3 ft. high, Upper Rupununi River, near Dadanawa, Lat. 2°45′ N., British Guiana, Jul. 24–29, 1922 (type, Herb. Field Mus.); *idem* 2284, growing 2 ft. high, Upper Mazaruni River, Long. about 60°10′ W., British Guiana, Sept. 22–Oct. 6, 1922 (Herb. Field Mus.).

In his Flora of The British West Indian Islands (p. 373. 1861), GRISEBACH stated that "a remarkable form, with all leaves simple, hispidulous achenia, and whitish-pubescent involucres, was collected by Dr. Alexander, but transitions into the common *B. leucanthus* [*i.e.*, *B. pilosa* var. *radiata* Schz. Bip.] occur among Mr. March's specimens." A former study of *Alexander's* specimens (Herb. Gray) left me unconvinced as to the value of their simple-leaved character for drawing taxonomic distinctions. The specimens recently collected by *De La Cruz* were obtained at different localities and about two months apart, yet both reveal in all respects a striking similarity.

[9] It is a pleasure to use here the name *Greenmania*, thus expressing, although all too inadequately, my very great indebtedness to my former teacher, DR. JESSE MORE GREENMAN. He it was who suggested, in 1912, that I study the genus *Bidens* and who rendered invaluable assistance to me during the initial stages of my research, particularly upon certain species and varieties of this section.

The leaves all are simple, thin, glabrous, the blades ovate-lanceolate, serrate, acute or subacuminate at apex, the larger ones about 3.5–4 cm. long and 1.5–2 cm. wide, the petioles slender and 1–2 cm. long. Compared with chance, more or less simple-leaved plants of *B. pilosa* proper that are sometimes found (*B. pilosa β discodea* Schz. Bip. *em.* I. *subsimplicifolia* O. Ktze. Rev. Gen. 1:322. 1891; "folia omnia vel plurima integra [cfr. Gris. Fl. Westindien]"-Ktze. *loc. cit.*) these plants appear to represent a more pronounced and less ephemeral form.

BIDENS OSTRUTHIOIDES var. **costaricensis** (Benth. *ex* Oerst.) comb. nov.; *B. costaricensis* Benth. *ex* Oerst., Kjoeb. Vidensk. Meddel. 1852: 94. 1852; *B. irazuensis* Calv. and Calv. Year Costa Rican Nat. Hist. xvi, 137 (plate), and 140. 1917. (pl. XXI) The well known *Bidens ostruthioides* (DC.) Schz. Bip. (*B. guatemalensis* Klatt) has "folia petiolata petiolis inferne hispido-ciliatis 1.5–2.5 cm. longis, petiolo adjecto plerumque subaequaliter 4.5–6.5 cm. longa, membranacea, infra multo pallidiora, tripartita (vel saepe summa indivisa); foliolis ovatis vel rhomboideo-ovatis, ciliatis, utrinque acute grosseque dentato-serratis; dentibus utroque latere 1–5, indurato-mucronatis."[10] A study of the type and other specimens of *B. costaricensis* shows the "folia petiolata petiolis inferne hispido-ciliatis 1–4 cm. longis, petiolo adjecto 5–12 cm. longa, acerrime bipinnatisecta vel plus minusve biternatisecta, foliolis segmentisve cuneato-lanceolatis, inciso-dentatis, dentium apice indurato-apiculatis et saepe mucronatis, margine sparsim ciliatis; supra ad venas plerumque minutissime creberrimeque glanduloso-setulosis, aliter glabris, in speciminibus exsiccatis saepe nigrescentibus; infra pallidioribus; lateralibus decurrentibus." (See footnote 10.)

In flower and fruit characters the two are identical. In size of plants, *B. costaricensis* may become taller, since *Lehmann* found it climbing to a height of 5 m., whereas *B. ostruthioides* has been reported several times as growing 0.3–1.5 m. high. Geographically, the former is more southern (growing, nevertheless, mostly at lower altitudes), ranging from the State of Oaxaca, Mexico, to Costa Rica,[11] while the latter ranges from the three Mexican States of

[10] *Ex meo chirographo ined.*

[11] Specimens of *B. ostruthioides* by *C. J. Graham* (Herb. Kew) from the region of the States of Mexico and Hidalgo (*fide* Benth. Pl. Hartweg. Pref. iv. 1839) exhibit, however, a strong approach in leaf division to *B. costaricensis*.

Michoacan, Mexico, and Vera Cruz to Guatemala. In Oaxaca both forms are common. Curiously, however, the *B. costaricensis* form, when found in Oaxaca, has been labeled at Gray Herbarium (*Pringle* 5848), the University of California Herbarium (*Purpus* 3109), and elsewhere as *B. ostruthioides*, while identical plants from Costa Rica have been referred very uniformly to *B. costaricensis*. A comparative study of all available material[12] indicates that *B. costaricensis* is best regarded as a variety of *B. ostruthioides*, from which it differs essentially in having the leaves somewhat larger and bipinnatisect or biternatisect, not merely tripartite.

Specimens examined: *Dr.* and *Mrs. P. P. Calvert*, forest below cinders, El Volcan Irazú, Costa Rica, Apr. 2, 1910 (Herb. Univ. Penn.); *C. W. Dodge* 3439, in the oak forest on the upper slopes, El Volcan Irazú, Cartago Province, Costa Rica, Aug. 18, 1925 (Herb. Gray); *C. Hoffmann* 105, higher mountain forest of El Volcan Irazú, May 6, 1855 (Herb. Berl.); *Dr. Otto Kuntze*, alt. 7000 ft., Cartago, Costa Rica, June 24, 1874 (Herb. N. Y. Bot. Gard.); *F. C. Lehmann* 119, alt. 6500 ft., climbing to 5 m. high, among shrubs and bamboos, west slopes of El Volcan Irazú, Mar. 28, 1878 (Herb. Mus. Vienna);[13] *idem* 1787, alt. 800 m., growing up to 1.5 m. high, in moist places, Rio Blanco, Costa Rica, Mar. 18, 1882 (Herb. Boiss.; Herb. Gray; Herb. Kew, 2 sheets); *Anders S. Oersted*, alt. 2000–5000 ft., Mt. Aguacate, Costa Rica, November, 1846 (type, Herb. Copenh.); *idem* San José, Costa Rica, 1845–1848 (Herb. Copenh., 2 sheets); *idem*, El Volcan Irazú etc., Costa Rica (*ex herb. Benth. in* Herb. Kew); *H. Pittier* 742, alt. 2800–3200 m., in oak forests of El Volcan Irazú, Dec. 12, 1888 (Herb. Gray); *idem* 14070, alt. 2300 m., Laguna del Reventado, El Volcan Irazú, Jan. 1, 1901 (Herb. Gray); *C. G. Pringle* 5848, alt. 9000 ft., Sierra de Clavellinas, State of Oaxaca, Oct. 26, 1894 (Herb. Gray); *C. A. Purpus* 3109, Cerro Verde, State of Oaxaca (in vicinity of San Luis Tultitlanapa, Puebla, near Oaxaca), July, 1908 (Herb. Berl.; Herb. Brit. Mus. Nat. Hist.; Herb. Univ. Calif.); *Charles L. Smith* 357, alt. 9000 ft., Sierra de Clavellinas, State of Oaxaca, Oct. 16–19, 1894 (Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.).

Coreopsis intermedia sp. nov.—Herba erecta, plus minusve glabra, forsitan perennis, supra parce ramosa, ±6 dm. alta, caule subtereti vel moderatim angulato, sulcato. Folia opposita, simplicia, basalia longe tenuissimeque petiolata petiolis usque ad 4.5 cm. longis, laminis oblongo-oblanceolatis vel anguste obtuseque obovatis; principalia caulina sessilia, late oblongo-lanceolata, ciliata, cras-

[12] I have examined 35 collections of *B. ostruthioides* and 14 of *B. costaricensis*.

[13] A form having petioles spiral and tendril-like in their lower ½ or ⅔, these being used by the plant in climbing.

siuscula, apice subacuta, 5–7 (−9.5) cm. longa et 1.2–2 (−3.2) cm. lata. Capitula pedunculata pedunculis 1.5–2.5 dm. longis, radiata, pansa ad anthesin 3.5–4 dm. lata et ±12 mm. alta. Involucri bracteae exteriores 8–10, lanceolatae vel lineari-lanceolatae, tergo glabratae, margine saepe diaphana ciliatae, apice acutae cartilagineaque, 4–7 (rariter −8) mm. longae; interiores late lanceolatae, plerumque 12–14 mm. longae. Flores ligulati circ. 8, flavidi, unicolores, obovati vel late oblanceolati, apice trilobati lobo mediano valde emarginato, circ. 1.5 cm. longi. Paleae superne elongatae et valde caudato-attenuatae. Florum disci stigmata apice caudata. Achaenia suborbicularia, valde obcompressa, dorsaliter convexa, brunneo-atra, alata alis membranaceis planis vel rarissime parce incurvatis 0.2–0.4 mm. latis, apice saepe bidenticulata, faciebus perspicue tuberculata, facie ventrali raro callosa, corpore ipso oblongo-obovato vel late oblongo-oblanceolato 2–3 mm. longa et 1.3–2 mm. lata.

Julian Reverchon 2077, in sandy woods, Mineola, Texas, June 12, 1900 (type in Herb. Berl.: cotype, Herb. Mo. Bot. Gard.); *idem* (*similiter*) 2077, sands, Mineola, Texas, June 10, 1900 (Herb. Mo. Bot. Gard.); *idem* 2041, sands, Big Sandy, Texas, May 27, 1901 (Herb. Mo. Bot. Gard., 2 sheets); *idem*, Pine Island, Texas, May 5, 1903 (Herb. Mo. Bot. Gard.).

The stems of the plants examined are uniformly leafy from bottom to top as in *Coreopsis pubescens* Ell.[14] and the leaves resemble the glabrous undivided ones occasionally found in that species. The elongate peduncles, however, also the pronounced differentiation between exterior and interior involucral bracts, are more as in *C. lanceolata* L.,[15] although not typical for that species.

COREOPSIS MUTICA **leptomera** var. nov.—Glabrata; e specie foliorum glabrorum segmentis tenuiter lanceolatis vel etiam linearilanceolatis differt.

[14] A species of the southeastern United States and ranging as far west as Missouri and Alabama.

[15] A species confined mostly to the eastern United States and southeastern Canada. Specimens have been collected, however, as far west as Wisconsin (*J. H. Schuette*, Door; Herb. Field Mus.), Iowa (*R. Burgess*, Clinton; Herb. Field Mus.), Arkansas (*H. E. Wheeler* 82, near Hazen, Grand Prairie; Herb. Field Mus.), and Texas (*F. Lindheimer*, west of Houston; Herb. Berl.: *W. F. Thurrow*, Hockley; Herb. Univ. Chicago). In China (Provinces of Chekiang, Kiangsi, Honan, etc.) *C. lanceolata* is introduced and now frequent, as shown by various herbarium specimens (Herb. Berl., Herb. Univ. Calif., etc.).

C. G. Pringle 9895, clay banks at altitude of 6800 feet, Dublan, State of Hidalgo, Mexico, Oct. 15, 1902 (type, Herb. Field Mus.: cotype, Herb. Berl.); *C. A. Purpus* 1339, Ixmiguilpan, State of Hidalgo, Mexico, September (Herb. Berl.).

The terminal leaflets are somewhat longer than the lateral and mostly measure 5–7 cm. long and 1.1–1.5 cm. wide. The species proper, which has wider leaf divisions, has been collected in the States of Hidalgo (*Ehrenberg* 354, Herb. Berl.; *C. G. Pringle* 8218, Herb. Berl., Herb. Field Mus.; *idem* 13041, Herb. Berl., Herb. Field Mus.; *C. A. Purpus* 1550, Herb. Field Mus.; *Rose*, *Painter*, and *Rose* 8837, U. S. Nat. Herb.) and Oaxaca (*C. Conzatti* 2074, Herb. Field Mus.); in Guatemala (*Heyde* and *Lux* 3792, Herb. Field Mus.; *W. A. Kellerman* 6296, Herb. Field Mus.; *H. von Tuerckheim* II. 2043, Herb. Field Mus., 2 sheets), etc. BLAKE (Proc. Amer. Acad. **49**:337. 1913; *cf.* Contr. Gray Herb. new ser. no. **52**:55. 1917) included *Pringle* 9895 under the species itself, but *Pringle* 9895 has foliage of very different aspect from that of DeCandolle's type (Herb. Deless.). This aspect is uniform for both sheets of the *Pringle* material cited, also for all eight of the very leafy branches of *Purpus* 1339 on the sheet cited for the Berlin Herbarium. The absence of intermediate forms, as also the previously known occurrence of two well marked varieties of *C. mutica* (var. *holotricha* Blake and var. *subvillosa* DC.), indicates that we have here another valid variety.

COREOPSIS TRIPTERIS **smithii** var. nov.—E specie foliis omnibus vel prope omnibus integris, lamina tenuiter oblongo-lanceolata differt.

John Donnell Smith, low, open woods near Montgomery, Alabama, Aug. 26, 1885 (type, Herb. Field Mus.); *T. H. Kearney, Jr.*, along Clear Creek, Bell County, Kentucky, September, 1893 (Herb. Field Mus.); *Charles Louis Pollard* 1222, Waynesboro, Mississippi, Aug. 8–9, 1896 (Herb. Field Mus.).

According to *Smith's* additional note on the type sheet, ASA GRAY had pronounced this plant a new variety of *Coreopsis tripteris* L. and, in a letter written in November, 1885, had given it a varietal name. While GRAY would probably have published the name had he not died shortly afterward, I am none the less constrained by the International Rules (Recomm. XIV.*e*) to pass over the name suggested by him and accordingly have named the type after its collector.

Coreopsis scopulorum sp. nov.—Frutex +2 dm. altus, glaber, ramosus, ramis foliosissimis, internodiis saepe tantum 3–5 mm. longis. Folia opposita, petiolata petiolis 6–10 mm. longis, petiolo adjecto tantum 1.2–2 cm. longa, plus minusve biternatisecta, segmentis ultimis linearibus, subcarnosis, margine saepe revolutis, apice mucronatis, vix 1 mm. latis. Capitula tenuiter pedunculata pedunculis 3–5 cm. longis ac saepe ad ramorum terminos 3–5-aggregatis, radiata (radiis in typo deficientibus sed circ. 8 ovariis sterilibus repraesentatis), demum circ. 8 mm. alta et 7–10 mm. lata. Stigmata disci florum apice perspicue caudata. Involucri bracteae exteriores circ. 8, anguste lineares, patenti-reflexae, apice rotundatae vel abrupte cuspidatae, circ. 3 mm. longae, interioribus lanceolatis atque apicem versus reflexis subaequalibus. Paleae perspicuae, late oblongae, apice rotundato-obtusae, achaeniorum corpora moderate superantes. Achaenia linearia, obcompressa, exalata, faciebus marginibusque erecto-setosa, atra, singula facie circ. 8-striata, corpore 5–6 mm. longa et 0.7–1 mm. lata, apice erecto-setosa ac perspicue biaristata aristis tenuibus erecto-hispidis circ. 2.5 mm. longis.

Edmund Heller, on cliffs at altitude of 7100 feet, summit of Mt. Garguez, British East Africa (Kenya Colony), Aug. 26, 1911 (type, U. S. Nat. Herb., no. 634308).

Apparently nearest *Coreopsis elgonensis* Sherff, from which it differs in its longer and petiolate leaves, its shorter external involucral bracts, its aristate achenes, etc.; and *C. chippii* Moss,[16] from which it differs in its much broader leaf-segments, etc. All three species offer a stronger resemblance to certain of the low, shrubby South American species of *Coreopsis* than to the various African species.

COREOPSIS JACKSONI **arthrochaeta** var. nov.—A specie foliis maximam partem dense hispidis, setis albidis elongatis sensim attenuatis multiloculatis differt.

R. L. Piemeisel and *L. W. Kephart* 166, at altitude of 3300 m., vicinity of Camp Gusisu, Aberdares Mts., British East Africa (Kenya Colony), Jul. 29, 1927.

[16] I am indebted to DR. ARTHUR W. HILL, Director of The Royal Botanical Garden of Kew, for his having sent me a photograph and type fragment of the very recently described *C. chippii*.

The species proper[17] is known to me through the type, collected by *Frederick J. Jackson*, Kikuyu region, British East Africa, 1889 (Herb. Brit. Mus.), also through *Dr. Edgar A. Mearns* 1291 and 1722, Mt. Kenia, British East Africa, September–October, 1909 (U. S. Nat. Herb.). The leaves are essentially glabrous. The material collected by *Piemeisel* and *Kephart* consisted of at least six small, entire plants and these offer a strikingly unique appearance because of their densely hispid leaves. Since endemism is pronounced among the plants of the Kikuyu region, and since elsewhere even where endemism is relatively unimportant (the United States) *Coreopsis* displays several forms that are accepted as varieties, it appears worth while to distinguish the *Piemeisel* and *Kephart* plants as connoting a group of varietal rank.

COREOPSIS TRIPTERIS **subrhomboidea** var. nov.—Folia tripartita foliolis lateralibus lanceolatis terminali rhomboideo-lanceolato 1.7–2.3 cm. lato, petiolo adjecto 6–8 cm. longa. Capitula minora, achaeniis tantum 4–4.5 mm. longis.

Ernest Jesse Palmer 29421, sandy, open woods bordering bog, near Texarkana, Bowie County, Texas, Oct. 27, 1925 (type, Herb. Gray).

In the more than two hundred collections of the species proper studied by me, all the leaflets were variously linear, oblong-linear or narrowly oblong-lanceolate, and the achenes were commonly 5–6 mm. long. In the *Palmer* plant the terminal leaflets approach very distinctly a rhombus in outline and the achenes are smaller, measuring only about 4–4.5 mm. in length.

COSMOS CARVIFOLIUS Benth. Bot. Voy. Sulphur 117. 1844; *Bidens carvifolia* (Benth.) Schz. Bip. *in* Seem. Bot. Voy. Herald 308. 1852–1857; *B. seemannii* Schz. Bip. *in* Seem. *loc. cit.* 307; *Cosmos*

[17] Formerly I had assumed (*cf.* BOT. GAZ. **81**:45. 1926) that the species was best interpreted as belonging in *Bidens*. The recent finding, however, of a mature specimen with numerous ripe achenes (*Mearns* 1722, U. S. Nat. Herb. no. 631676) showed that there had been no warrant for transferring the species to *Bidens*. The mature achenes of the species proper are seen to be blackish or brownish-black, thick-clavate, more or less swollen and quadrangulate, not visibly striate, about 3.2–3.7 mm. long and 1.1–1.7 mm. thick, glabrate, exalate and exaristate but at the apex often accompanied by a basal remnant of the withered disc floret (giving it the appearance of having a short thick beak). This last character is unknown to me elsewhere either in *Bidens* or in *Coreopsis*, although of course in certain related genera (e.g. *Heterosperma*) some achenes have a true beak or neck looking somewhat like the disc floret's basal remnant here.

seemannii (Schz. Bip.) Gray, Proc. Amer. Acad. **19**:16. 1883; *etiam ex* Greenm., *ibid.* **41**:265. 1905; *Bidens seemanii* Schz. Bip. *ex* Sherff, BOT. GAZ. **64**:28. 1917 (sphalm).—The type of this species was collected by *Mr. George Barclay*, of the Sulphur Expedition, at Tepic, Territory of Tepic, Mexico. In herbaria, however, the various additional specimens collected later in the same and other regions of Mexico have been referred quite uniformly to *C. seemannii* (Schz. Bip.) Gray. The types of both *C. carvifolius* and *C. seemannii* are still preserved at Kew and are seen to be identical. The former, however, is of a plant that has a somewhat irregular growth habit, with more than the usual number of branches and the more numerous heads rather stunted in size. BENTHAM compared his type with *C. bipinnatus* Cav., a species having 2- (rarely 3-) aristate achenes, while his type had 5-aristate achenes. More commonly the achenes are 6–8-aristate.[18]

Specimens examined: *Brother Arsène*, Cerro San Miguel, Morelia, State of Michoacan, February, 1909 (Herb. Deless.; Herb. Field Mus.; Herb. Phila.); *George Barclay* (Voyage of the Sulphur), Tepic, Territory of Tepic, 1836–1842 (type, Herb. Kew); *Dr. Ghiesbreght* 264, cold ground, September–October (Herb. Gray, 2 sheets); *Ynes Mexia* 601, common at alt. 1000 m., on open hillside, road from Tepic to Jalcojotan, State of Nayarit, Sept. 15, 1926 (U. S. Nat. Herb.); *Dr. Edward Palmer* 1852, Tepic, Territory of Tepic, Jan. 5–Feb. 6, 1892 (Herb. Field Mus.; Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.); *C. G. Pringle* 8845, alt. 5000 ft., in fields, Uruapan, State of Michoacan, Oct. 8, 1904 (Herb. Carnegie Mus.; Herb. Deless.; Herb. Gray; Herb. Phila.; Herb. U. S. Nat.); *Joseph N. Rose* 3435, in the Sierra Madre, near Santa Teresa, Territory of Tepic, Aug. 11, 1897 (U. S. Nat. Herb.); *Berthold Seemann* 2014 (Voy. of the Herald), Sierra Madre, Mexico (Herb. Gray; Herb. Kew; type collection of *Cosmos seemannii*).

Cosmos gracilis sp. nov.—Herba annua, erecta, glabra, gracilis, infra simplex supra ramosa, 4–6 dm. alta, caule subtetragono, internodiis quam foliis multo brevioribus. Folia tenuiter petiolata petiolis parce marginatis usque ad 1.5 cm. longis, petiolo adjecto 4–6 cm. longa, bipinnatisecta, segmentis membranaceis saepius oblongo-linearibus, margine subciliatis, apice acutis, plerumque 1–3 mm. latis. Capitula tenuiter pedunculata pedunculis usque ad 16 cm. longis, non numerosa (unicae plantae circ. 5–12), radiata, pansa ad

[18] *Palmer* 1852, from the type locality of Tepic, has the achenes mostly 5-aristate as in BENTHAM'S type. These are accompanied however by a few achenes with 6 aristae and several with only 4.

anthesin circ. 1.8 cm. lata et circ. 1 cm. alta. Involucri glabri bracteae exteriores 5–7, anguste cuneato-lanceolatae, obsolete nervatae, saepe patentes, tantum 1.5–2.2 (raro –4) mm. longae; interiores oblongo-lanceolatae demum circ. 7–8 mm. longae. Flores ligulati circ. 5 vel 6, flavidi (non aurantiaci), ligula obovati, apice truncato-lobulati, tantum circ. 6–8 mm. longi. Paleae lineari-lanceolatae. Achaenia circ. 6–10, fusiformia, rotundato-tetragona, unaquaque facierum 1-sulcata, infra nigra glabrataque supra rostrato-angustata ac straminea erecto-hispidulaque, apice spinulis patentibus minutissimisque coronata vel demum calva, 1.1–1.7 cm. longa et circ. 1.1–1.3 mm. crassa.

Henri François Pittier 5053, at altitude of 100–350 meters, vicinity of Olá, Province of Coclé, Panama, Dec. 7–9, 1911 (type, U. S. Nat. Herb.: cotype, Gray Herb.).

Differs from *Cosmos sulphureus* Cav. in having smaller size, more delicate habit, the stem glabrous with the internodes shorter than the leaves (these smaller and less compound), the flowering and fruiting heads much smaller, the exterior involucral bracts proportionately smaller, the rays golden yellow not orange, etc.

COSMOS DIVERSIFOLIUS **pumilus** var. nov.—Herba pumila, 3–4 dm. alta, foliis basalibus, pedunculis scaposis, capitulis pansis ad anthesin tantum circ. 4 cm. latis, radiis subalbidis et parce violaceis, achaeniis atris erostratis corpore tantum 6–10 mm. longis et 0.7–1 mm. crassis, apice plerumque 2- (raro usque ad 4-) aristatis aristis tantum circ. 1–1.5 mm. longis.

Carl Albert Purpus 3029, in meadows, Boca del Monte, State of Puebla, Mexico, September, 1908 (type, Herb. Field Mus.: cotypes, Herb. Mo. Bot. Gard.; Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.).

COSMOS OCELLATUS **greenmanii** var. nov.—A specie ipsa foliis plerumque tripartitis foliolis ovato-lanceolatis serratisque, achaeniis etiam juvenibus apice exaristatis differt.

Cyrus Guernsey Pringle 8386 *pro parte*, at altitude of 7500 feet, in thin soil on the knobs of the Sierra de Tepoxtlan, State of Guerrero, Mexico, Oct. 14, 1900 (type, Herb. Field Mus.: cotypes, Herb. Mo. Bot. Gard., etc.).

Named in tribute to DR. JESSE MORE GREENMAN, Curator of the Herbarium of the Missouri Botanical Garden (*cf.* footnote 9). The species proper, originally described by GREENMAN from other speci-

mens by *Pringle* (under the same number), has usually 2–3-pinnate leaves, achenes 2-aristate, etc.

COSMOS PEUCEDANIFOLIUS Wedd. Chlor. And. 1:70. 1855; *Bidens peucedanifolius* (Wedd.) O. Ktze. and vars. *bipinnatisecta* O. Ktze. and *soratensis* O. Ktze., Rev. Gen. 3 II:137. 1898.—In the past, several students of South American Compositae have regarded *Cosmos diversifolius* Otto, a species originally described from Mexican material, as extending from Mexico into Peru and Bolivia. A comparative study of many specimens from northwestern South America and from Mexico shows, however, that the former, while differing among themselves considerably, are fairly well marked and can usually be distinguished from *C. diversifolius* without much difficulty.

WEDDELL (*loc. cit.*) was the first to segregate the South American material. He described three species—*C. integrifolius*, *C. peucedanifolius*, and *C. subpubescens*. The first and last of these are treated in the immediately following sections of this article. His *C. peucedanifolius*, from Bolivia, may best be recognized by the narrowly linear leaf segments: "foliis profunde pinnatisectis, lobis utrinque 2 anguste linearibus elongatis integris, terminali caeteris fere duplo longiore. . . ." (Wedd. *loc. cit.*). OTTO KUNTZE'S vars. *bipinnatisecta* and *soratensis* are represented by good specimens still extant (Herb. Berl.; Herb. N. Y. Bot. Gard., etc.) and are seen to belong here, differing only in the here unimportant matter of degree of leaf dissection.

Specimens examined: *Miguel Bang* 1302, vicinity of Sorata, Bolivia, May 1892 (Herb. Gray; Herb. Mo. Bot. Gard.; Herb. N. Y. Bot. Gard.; Herb. Phila.; Herb. U. S.Nat.; Herb. Univ. Vienna; type collection of *Bidens peucedanifolia* var. *soratensis* O. Ktze.); *Dr. Otto Buchtien* 611, alt. 3200 m., mountain slopes, Unduavi, North Yungas, Bolivia, February, 1915 (Herb. Field Mus.; Herb. U. S. Nat.); *Cardenas* "7 special," Cochabamba, Bolivia, 1922 (Herb. U. S. Nat.); *K. Fiebrig* 2966, alt. 3300 m., Patanca, southern Bolivia, Feb. 1, 1904 (Herb. Gray); *Dr. Otto Kuntze*, alt. 3000 m., Rio Juntas, Bolivia, April, 1892 (Herb. N. Y. Bot. Gard.; cited by *Kuntze* for his *Bidens peucedanifolia* var. *bipinnatisecta*); *idem*, alt. 3400 m., Tunari, Bolivia, May 4, 1892 (Herb. Mo. Bot. Gard.; Herb. N. Y. Bot. Gard.; Kuntze's first cited specimen of his var. *bipinnatisecta*); *Dr. H. H. Rusby* 1682 *pro parte*, alt. 10000 ft., Sorata, Bolivia, February, 1886 (Herb. Mo. Bot. Gard.; Herb. N. Y. Bot. Gard.); *C. H. T. Townsend* 1507, Pachicayo, Peru, Mar. 27, 1913 (Herb. U. S. Nat.).

COSMOS PEUCEDANIFOLIUS var. **cochabambensis** (O. Ktze.) comb. nov.; *Bidens peucedanifolia* var. *cochabambensis* O. Ktze. Rev. Gen. 3 II:137. 1898; *Cosmos integrifolius* Wedd. Chlor. And. 1:70. 1855 (*ex descript. et patr.*).—*C. integrifolius* apparently was segregated from *C. peucedanifolius* mainly because of its leaf characters: "foliis lineari-lanceolatis, decimetralibus, integerrimis rariusve lobo lineari ad unum alterumve latus vel utrinque instructis. . . ." (Wedd. *loc. cit.*). The type collection of KUNTZE's var. *cochabambensis* (*Bang* 1021) shows a slight tendency toward greater division of the leaves, but otherwise agrees with WEDDELL's description of *C. integrifolius*. KUNTZE's interpretation of this form, as representing a variety, is doubtless nearer the truth than was WEDDELL's.

Specimens examined: *Miguel Bang* 1021, vicinity of Cochabamba, Bolivia, 1891 (Herb. Gray; Herb. Mo. Bot. Gard.; Herb. Phila.; Herb. U. S. Nat.; type collection).

COSMOS PEUCEDANIFOLIUS var. **tiraquensis** (O. Ktze.) comb. nov.; *C. subpubescens* Wedd. Chlor. And. 1:70. 1855 (*ex descript. et loco*); *C. pulcherrimus* Schz. Bip., Bull. Soc. Bot. Fr. 12:79. 1865 (*nomen subnudum*); *Bidens pulcherrima* Schz. Bip., Linnaea 36:528. 1865–1866 (*nomen subnudum*); *Cosmos marginatus* Klatt, Abhandl. Naturf. Ges. Halle 15:328. 1882; *Bidens peucedanifolia* var. *tiraquensis* O. Ktze. Rev. Gen. 3 II:137. 1898.—WEDDELL'S *C. subpubescens* was based on a plant by *Gay* from the Province of Cuzco, Peru. A careful reading of his description shows that here again the leaves were the chief basis of distinction: "foliis pinnatisectis, lobis utrinque 2–3-lanceolatis integris vel pauci-dentatis mucronatis. . . . ." There have come from Cuzco and the adjacent region many specimens which match WEDDELL's description fairly well. One collection was by *A. Mathews* from the Province of Chachapoyas, Peru. This was the basis of *Cosmos marginatus* Klatt. Two others, by *G. Mandon* and both under his no. 54, were from the vicinity of Sorata, Bolivia, and were the basis of the names (*sine descript.*) *Cosmos pulcherrimus* Schz. Bip. and *Bidens pulcherrima* Schz. Bip. Another was by *Dr. Otto Kuntze* from Tiraqui, Bolivia. This was the basis of *Bidens peucedanifolia* var. *tiraquensis* O. Ktze. It is through this form, which cannot be regarded as of higher than varietal rank, that

C. peucedanifolius makes its nearest approach to low and rather dwarfed forms of *C. diversifolius* Otto.[19]

Specimens examined: *Mrs. Adolph F. Bandelier* 18, alt. 12500 ft., Isl. Titicaca, Lake Titicaca, Bolivia, 1905 (Herb. N. Y. Bot. Gard.; *nomen incolarum aymaranarum* Panti-Panti); *Dr. Otto Buchtien* 3074, alt. 3840 m., Isl. del Sol, Lake Titicaca, Bolivia, March, 1910 (Herb. Field Mus.; Herb. N. Y. Bot. Gard.; Herb. U. S. Nat.); *K. Fiebrig* 2822, alt. 3000 m., Pinos near Tarija, southern Bolivia, Jan. 22, 1904 (Herb. Deless.; Herb. Field Mus.; Herb. Mo. Bot. Gard.); *F. L. Herrera*, alt. 3000–3600 m., Cuzco, Peru, July, 1923 (Herb. U. S. Nat.); *idem* 1025, alt. 3700 m., Hacienda Churu, Province of Paucartambo, Peru, January (*e pittacio lectoris ipsius*), 1926 (Herb. Field Mus.; Herb. Gray, 2 sheets; Herb. Mo. Bot. Gard.; Herb. U. S. Nat.; *nom. incolarum* Panti); *Dr. Otto Kuntze*, alt. 4000 m., Tiraqui, Bolivia, Apr. 1–4, 1892 (type, Herb. N. Y. Bot. Gard.); *G. Mandon* 54, alt. 2650–3000 m., in uncultivated places, thickets, etc., vicinity of Sorata, along road to Lucatia, Bolivia, February–March, 1858 (Herb. Deless., 3 sheets; Herb. N. Y. Bot. Gard.); *idem* (*similiter*) 54, alt. 2800–3000 m., in thickets everywhere, vicinity of Sorata, January–March, 1859 (Herb. Deless.; Herb. Gray; Herb. N. Y. Bot. Gard.); *Alexander Mathews*, Province of Chachapoyas, Peru (Herb. Gray, 2 sheets; type material of *C. marginatus* Klatt); *Dr. H. H. Rusby* 1682 *pro parte*, alt. 10000 ft., Sorata, Bolivia, February, 1886 (Herb. Gray; Herb. N. Y. Bot. Gard., *cum spec. ipsa;* Herb. Phila.; Herb. U. S. Nat.); *A. Weberbauer* 7597, alt. 3600 m., on grass steppe with scattering shrubs, Yanahuajra Valley, Department of Ayacucho, Province of Huanta, Peru, Mar. 18, 1926 (Field).

CHICAGO NORMAL COLLEGE
CHICAGO, ILL.

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EXPLANATION OF PLATES XVII–XXI

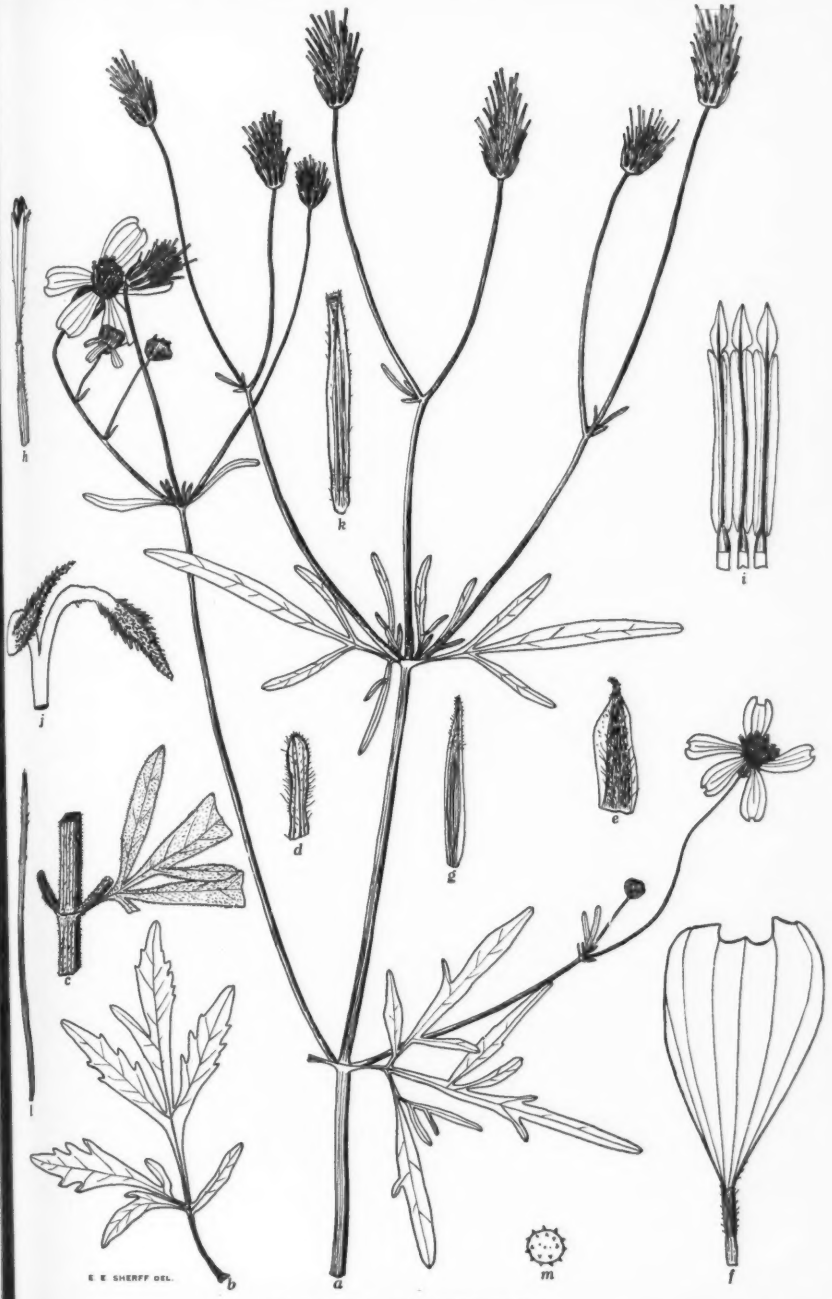
PLATE XVII

Bidens pilosa var. *bimucronata* f. *odorata* (*B. inermis* Wats.): *a*, flowering and fruiting branch, ×0.65; *b*, lower cauline leaf, ×0.65; *c*, portion of stem magnified to show details, ×2; *d*, exterior involucral bract, ×4; *e*, interior involucral bract, ×4; *f*, ray floret, ×4; *g*, palea, ×4; *h*, disc floret, ×4; *i*, anthers, ×20; *j*, style branches, ×20; *k*, *l*, achenes, ×4.6; *m*, pollen grain, ×385; all from *C. G. Pringle* 1291, Herb. Field Mus.

PLATE XVIII

Bidens coriacea: a, flowering and fruiting branch, ×0.62; *b*, exterior involucral bract, ×2.6; *c*, *d*, *e*, various interior involucral bracts, *c*, *e*, ×2.6, *d*,

[19] My present treatment is necessarily abridged. Further details are reserved for my forthcoming *Revision of the genus Cosmos*.



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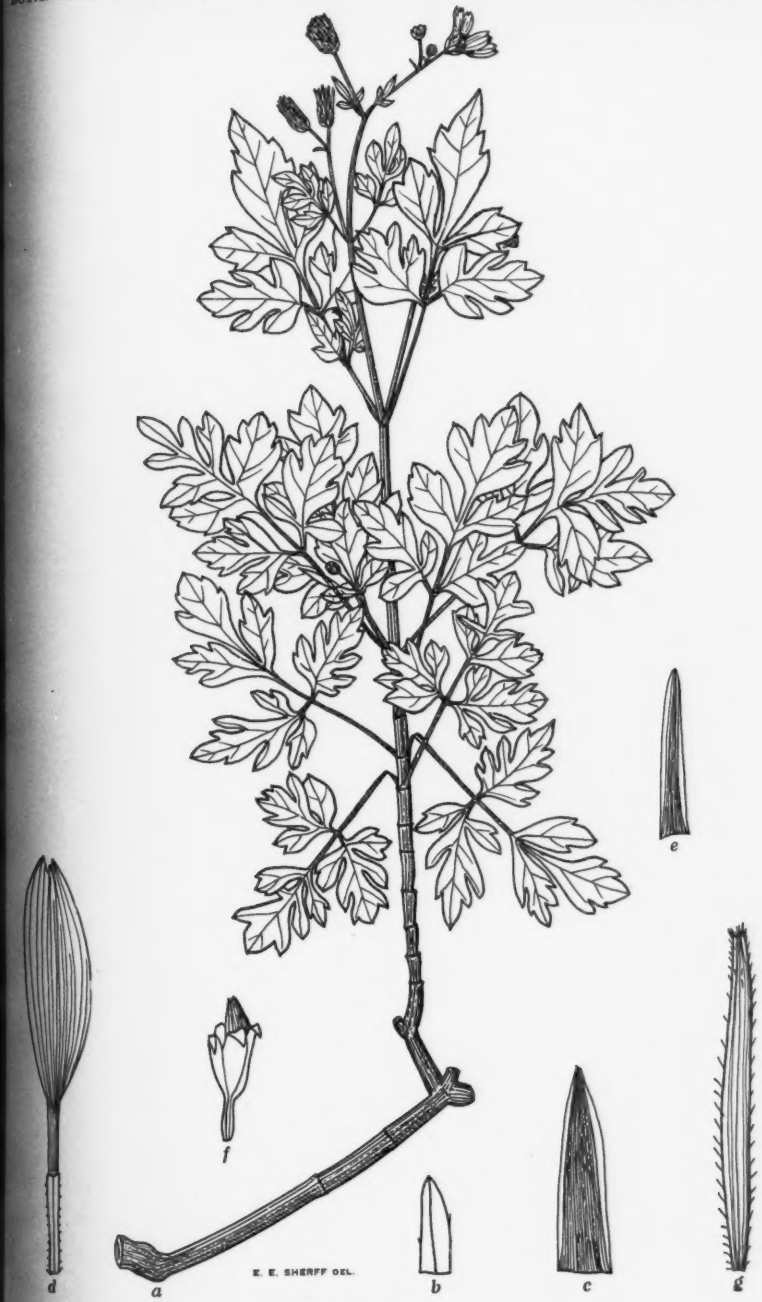

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×1.9; *f*, ray floret, ×1.9; *g*, palea, ×1.9; *h*, disc floret, ×3.9; *i*, *j*, achenes, ×3.9; *k*, achene, ×1.9; *a*, from *Mr. J. D. Snowden* 28, alt. 4700 ft., Uganda, British East Africa, 1913, Herb. Kew; *b*, *d*, *f*, *g*, *h*, *i*, *j*, from *G. F. Scott Elliot* 7410, cotype of *Bidens ruwenzoriensis* (S. L. Moore) Sherff in Herb. Kew; *c*, *e*, *k*, from *Fischer* 367, type of *B. coriacea* in Herb. Berl.

PLATE XIX

Bidens schizoglossa: a, flowering and fruiting branch, ×0.68; *b*, exterior involucral bract, ×6.8; *c*, interior involucral bract, ×6.8; *d*, *e*, rays, ×6.8; *f*, palea, ×6.8; *g*, disc floret, ×6.8; *h*, *i*, achenes, ×6.8; all from *W. A.* and *C. B. Setchell*, near Huehue, Isl. Hawaii, June 24, 1924, type in Herb. Univ. Calif.

PLATE XX

Bidens obtusiloba: a, flowering and fruiting branch, ×0.74; *b*, exterior involucral bract, ×7.4; *c*, interior involucral bract, ×7.4; *d*, ray floret, ×7.4; *e*, palea, ×7.4; *f*, disc floret, ×7.4; *g*, achene, ×7.4; all from *D. LeRoy Topping* 2941, type in Herb. Univ. Calif.

PLATE XXI

Bidens ostruthioides var. *costaricensis: a*, *b*, flowering branches, ×0.68; *c*, exterior involucral bract, ×4; *d*, interior involucral bract, ×4; *e*, ray floret, ×2.7; *f*, palea, ×4; *g*, disc floret, ×4; all from sheet of assorted *Oersted* materials from Mt. Irazú, Mt. Aguacate, etc., in BENTHAM's herbarium in Herb. Kew.

PHYSIOLOGICAL IMPORTANCE OF CALCIUM IN LEGUME INOCULATION

W. A. Albrecht and F. L. Davis

(with four figures)

Introduction

That calcium should be an important factor in the nodulation of legumes on acid, or sour, soils is suggested by the varying results in nodulation obtained on these soils. This is indicated especially in the soils of northeastern Missouri and southern Illinois, where the many or even frequent and repeated failures of inoculation with pure cultures have been found to occur on certain predominating acid soil types; while improved inoculation results when these soils are supplied, either previous to or at the time of planting, with limestone, acid phosphate, or other calcium-bearing materials.

Data presented by Hellriegel and Wilfarth, in their original article (9), establishing the relation between legume root nodules and nitrogen fixation, show a stimulating effect of calcium upon nodulation and growth of serradella. Recently Alway (1), in comparing the effectiveness of soil transfer with that of pure cultures as inoculation for alfalfa on lime-deficient, sandy soils, found that when the land had not been limed the soil transfer method was far more effective for the first seeding. Excessive increases in the amount of culture did not make this method as effective as soil transfer. On land limed well in advance of seeding, however, the inoculations by soil transfer and by the pure culture method were of equal efficacy. This seems to indicate an inability of the organisms to establish themselves quickly in a lime-deficient soil habitat.

Fellers (8), in a summary of his work on the factors affecting nodulation of soy beans, states: "The bacterial infection of roots does not take place readily on acid soils even when the root infecting organisms are plentiful in the soil." Bryan (3), in a study of the effect of acid soil reactions on nodulation of soy beans, found that in general the hydrogen-ion relations for the organism tend to be the

same as those for the host plant. He secured a maximum nodulation at pH 6.5, and none below pH 4.9, although the critical hydrogen-ion concentration for the organisms in solution cultures was found to be pH 3.5–3.9.

TRUOG (15) ventured the theory that the effect of soil reaction on the activity of the bacteria within the nodule is indirect, since the environment of the bacteria when in the nodule must be that of the plant tissue. KARRAKER (11), in examining this viewpoint, found that the root system of a single alfalfa plant, divided between a lime-deficient and a limed soil, gave differences in nodule formation corresponding to those obtained on different plants growing wholly within these different soils. He concluded that the effect of soil reaction on nodule formation must be one of localized character in the plant, a direct effect of soil pH on the bacteria in the nodules, or an antecedent effect of the soil acidity on the bacteria, while they are existing non-symbiotically in the soil.

SCANLAN (14) concluded that hydrogen-ion concentration has no direct effect upon inoculation, and analogous inoculation resulted from the use of limestone and calcium acetate where the former lessened the hydrogen-ion concentration while the latter had no effect upon it.

FALK (7) thinks that neutralization of the acid is not the only effect of certain valuable salts, this being indicated by the fact that magnesium carbonate and phosphate improve bacterial growth more effectively than calcium carbonate. MACHIDA (12) demonstrated that calcium salts and not magnesium salts are effective in protecting bacteria against lethal agencies. This work was substantiated by CHAMBERS and REZINKOFF (5), studying the protoplasm of *Amoeba proteus*.

WINSLOW and FALK (16) found that concentrations of 0.4 per cent of sodium chloride alone, or of 0.2 per cent of calcium chloride alone, exerted marked lethal effects upon the growth of a typical colon bacillus, *B. communis*, while, a mixture of these two, in the ratio of one Ca-ion to five Na-ions and in concentrations as high as 5 per cent of the salt, was actually beneficial in its effect upon the growth of the organism. HOTCHKISS (10) made a survey of the effects of cations upon the bacterial growth of *B. coli*, and found that

calcium chloride in a 0.5 molar concentration limited growth completely, but stimulated growth in a 0.05 molar concentration. SCANLAN (14) found that one part of calcium chloride to 1500 parts of solution was the optimum concentration for stimulating growth and longevity of *B. radicicola*.

Investigations to date give numerous observations on the effects of limed and acid soils on nodulation of legumes, with almost as many theories as to the operating causes. These conditions emphasize the need for a fuller understanding of the fundamental facts controlling the responses in nodule production by legumes and their bacteria on soils of varying degrees of soil acidity or base deficiency.

Experimentation

PART I

The work here reported bears testimony to some of the preceding viewpoints. Results analogous to those of KARRAKER were obtained in working with soy beans on an acid Putnam silt loam. Seedlings were grown in sterile sand for 10-14 days, when the tap roots were cut off just below the lateral roots, which had developed in good numbers and to a length of about 1 inch. These seedlings were placed over a water-tight partition in a container with one-half of the root system carefully planted into an acid soil (pH 5.14) on one side, and the other half into the same soil after it had been thoroughly mixed with calcium carbonate at the rate of 8000 pounds per two million of soil. Liberal quantities of a suspension of inoculating bacteria were added as the soil was filled into the pans around the root system. Five seedlings with their lateral roots so divided were set into each container. In addition, ten seedlings, with their tap roots likewise cut off, were planted into the container, five on the side of the acid soil and five on the side of the limed soil. Water was maintained at the optimum by daily surface applications.

Although there was a high mortality of plants in consequence of tap root pruning and replanting, those that lived grew very satisfactorily. At the end of 5 weeks they were carefully taken up and the nodules counted. The nodule counts of the plants with divided roots and checks are summarized in table I. The results show an increase of 208 per cent in the number of nodules formed on the portion of

the roots growing in the calcium soil as compared with those formed on the portion in the untreated soil. The difference of 181.1 per cent in nodulation by the check plants, grown wholly within one kind of soil, is approximately the same ratio. The comparison of the degrees of inoculation of the divided root plants and the checks is made on this basis rather than on the actual plant units, because the average nodulation of either portion of the root of the divided root plants represents but one-half of the normal nodulation of the plants.

TABLE I

NODULATION OF PLANTS GROWN WITH PART OF ROOT SYSTEM IN CALCIUM-TREATED SOIL AND PART IN UNTREATED SOIL (pH 5.14)

TREATMENT	PLANTS WITH DIVIDED ROOTS			CHECK PLANTS			
		Nodule production		Calcium-treated soil		Untreated soil	
	No. of plants	Roots in calcium-treated soil	Roots in untreated soil	No. of plants	No. of nodules	No. of plants	No. of nodules
Uninoculated	4	0	0	6	0	5	0
Inoculated	23	160	77	28	494	31	302
Average per plant		6.95	3.34		17.64		9.74
Percentage increase		208.0			181.1		

The reliability of these data is questionable on account of, first, the small number of plants on which the test was completed, and second, the unequal development of the two parts of the divided root systems.

The results obtained for soy beans agree well with those for alfalfa by KARRAKER (11). They indicate that, if the effect of the calcium is a physiological one through the plant, this effect is local in character, and the calcium is certainly not translocated to all the roots of the plant for equal effectiveness in improving inoculation; or, that the depressed nodulation in this acid soil is due to an effect of the soil conditions upon the bacteria before they have infected the roots of the host plant.

PART II

A test of the stimulating effect of calcium upon nodulation of soy beans on an already well inoculated soil emphasized further the possible physiological effect of this element. The soil used had a pH

of 5.5, and was well stocked with the soy bean organism in consequence of well inoculated crops of soy beans on three previous seasons.

Enough of this soil was mixed with calcium carbonate at the rate of 8000 pounds per two million to fill thirty pots, while a like number were filled with untreated soil, and each of the sixty pots was planted with five sterile, sprouted soy beans. After cautious culture for five weeks, when the plants showed a uniform healthy growth with slight superiority in color, size, and appearance in the case of those given calcium carbonate, counts of the nodules were made as given in table II. The results show an increase of 336 per cent in the numbers of nodules as a result of liming, in spite of the fact that

TABLE II

NODULE NUMBERS AS INFLUENCED BY CALCIUM CARBONATE ON SOIL GROWING SOY BEANS PREVIOUSLY*

SOIL TREATMENT	POTS	PLANTS	NODULE NUMBERS				
			Total	Range per pot	Average per pot	Average per plant	Increase per cent
None	30	130	1659	10–142	55.3	12.0	
Calcium carbonate	30	133	5353	64–380	178.4	40.2	336.8

* Silt loam soil pH 5.5.

the soil was already well inoculated with the organisms. This agrees with FELLERS' observation, and while it demonstrates the correlation of nodulation of legumes on acid soils with the acidity, it does not establish the relation of cause and effect between them.

PART III

In order to differentiate more closely between the effect of soil reaction upon nodulation and the importance of calcium to nodulation, a study of the effect of calcium, as calcium chloride, on the viability of *B. radicicola* was made. For this a rather well isolated soil, mainly of residual formation from limestone, was collected from a timbered area. It was decidedly acid (pH 5.4), and sterile with reference to the soy bean organism. Sixty pots (3.5 inches in diameter) of this soil were set up, planted to soy beans, and given thorough inoculation with cultures of bacteria. One-half of these pots

were given the additional treatment of a solution supplying 88 mg. of calcium chloride per pot, the calcium equivalent of 2000 pounds of calcium carbonate per two million of soil. Eight additional pots without inoculation or calcium treatment were used as checks. The nodule counts were made after growth of five weeks.

Not a nodule had formed on a single plant in the untreated soils, and but three single nodules on as many plants in the calcium-treated soil, although a good growth of plants was obtained in all the pots. In order to test the soil for the presence of the organism, sixteen pots of each of the treated and untreated soil were immediately replanted with sprouted beans. No further inoculation or treatment was added. Again after five weeks of growth these were

TABLE III

NODULE NUMBERS OF SOY BEANS AS INFLUENCED BY CALCIUM CHLORIDE TREATMENT ON SOIL

	Treatment	Pots	Plants	Sterile plants	Inoculated plants	Total nodules
Inoculated first planting	None	30	130	130	0	0
	Calcium chloride	30	133	130	3	3
Uninoculated second planting	None	16	72	69	3	3
	Calcium chloride	16	80	0	80	146

taken up and the nodules counted as before. The data are summarized in table III.

The results of this trial emphasized the difficulty of establishing the soy bean organism within this soil by a single inoculation, and demonstrated that even though the organism did not exist in the untreated soil, it continued its existence in the calcium-treated soil despite no significant change in the soil reaction. This indicates, in substantiation of SCANLAN'S findings, that the response by the organism must be due to effects by the calcium.

PART IV

Since calcium seemed to favor nodulation, an attempt was made to determine whether or not this influence comes in consequence of its direct effect on the soil conditions, or of an indirect effect through

the plant. Quartz sand was treated with hydrochloric acid for 3 hours, washed with tap water and then with distilled water until the test for chlorides was negative. The sand was dried, and heated at 111° C. for 48 hours for drying and for sterilization. Part of the sand was treated with calcium carbonate at the rate of ten thousand pounds per two million pounds of sand.

Sterilized soy beans, the progeny from a single plant, were germinated for 24 hours, planted in both the limed and unlimed sand, and then grown for 10 days. Seedlings from both the calcium-bearing and the calcium-deficient sands were washed free of adhering particles and transplanted into the respective halves of each of two

TABLE IV

NODULE PRODUCTION BY SOY BEANS IN NEUTRAL AND ACID SOILS AS INFLUENCED BY CALCIUM IN TRANSPLANTED SEEDLINGS

KIND OF SOIL	pH	SEEDLING TREATMENT	NO. OF PLANTS	NODULE NUMBERS		CALCIUM CONTENT (MG. CA)		
				Range per plant	Average per plant	Seedlings	Soil	Seeds
						Per 100	Electrodialysable per 10 gm. W-free	Per 100
Neutral	7.8	None	60	12–77	36.6	17.07	24.07	6.85
		Calcium	67	9–67	38.9	30.14		
Acid	5.5	None	69	1–7	3.4	17.07	11.78	6.85
		Calcium	79	2–25	15.1	30.14		

flats of well prepared soils. One of these soils was a lime-deficient field soil and the other a neutral garden soil, both of which had grown well-inoculated soy beans during the three preceding seasons. Excellent growth resulted in both flats, and after 5 weeks the plants were removed and counts made of the nodules, with the results given in table IV.

On the lime-deficient soil the calcium-starved plants developed few nodules. In marked contrast to this, there were almost five times as many nodules within this soil in consequence of allowing the seedlings to grow in calcium-bearing sand and to transplant their needed calcium to the lime-deficient soil. On the neutral soil no differences in nodulation occurred as a result of either treatment to the seedlings.

In order to gain some additional information, the following chemical determinations were made: (1) the calcium content of the bean seeds, of the 10-day old seedlings grown on the calcium-deficient sand, and of those grown on calcium-bearing sand, according to a modification of McCRUDDEN'S (13) method; (2) the total dialysable base of both soils, by BRADFIELD'S (2) method; and (3) the total dialysable calcium in these soils by the same method. The summary of these analytical data is given also in table IV, with the omission of total dialysable base.

From the complete data in table IV it will be seen that the soil on which good nodulation occurred, regardless of seedling treatment, had a pH of 7.8 and gave 24.07 mg. electro-dialysable calcium per 10 gm. of soil; while the soil which gave good inoculation only on calcium-treated seedlings had a pH of 5.5 and 11.78 mg. of electro-dialysable calcium per 10 gm. of soil.

The increased nodulation of the higher calcium-containing seedlings on the calcium-deficient soil demonstrates that the presence of calcium in the plant increases nodulation of soy beans on such a soil. On the other hand, the lack of difference in nodulation of the seedlings on the more calcium-sufficient soil indicates that either the presence of calcium in the plant does not affect nodulation on such a soil, or that if it does, the calcium-starved plants take calcium from the soil soon enough to offset the measurable effect in difference in nodulation.

The increase in calcium content of the seedlings grown on the acid-extracted sand over the calcium content of the bean seeds was due to the calcium that was carried back into the acid-extracted sand by the tap water with which the sand was washed. Although the calcium content of the seedlings grown on this extracted sand was more than expected from the analysis of the seeds, the actual amount contained was but little more than half that of the seedlings grown in the calcium-bearing sand. Improved methods on this point will probably intensify the results obtained.

PART V

After finding that the presence of calcium within the plant increased the nodulation of soy beans on an acid lime-deficient soil, a

microchemical study of the seedlings was made to locate, if possible, any histological differences in the calcium-starved and calcium-fed seedlings. Parts from both stems and roots of each of these were collected at the age of 10 days, and prepared by the usual method for sectioning in paraffin. Micro-photographs of cross-sections of the stem taken near the plant crown are reproduced in figs. 1–4. These photographs are representative sections from more than forty slides. The differences shown in the figures were the same throughout the

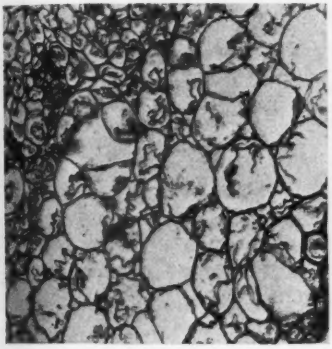
FIGS. 1, 2.—Cross-section of stems of calcium-starved and calcium-bearing soy bean seedlings (10 days old): fig. 1, calcium-bearing, fig. 2, calcium-starved; ×170.

slides. The cell walls of the calcium-starved seedlings failed to retain their shape, and apparently gave way before the microtome blade, while the cells of the calcium-fed plants stood up under the treatment and gave distinctly better sections.

These differences were innate to the material, since the comparative sections of calcium-starved and calcium-fed seedlings were cut at the same time, and on the same microtome. The materials were fixed and processed as separate samples, but in duplicate of the same lot, so that variation in laboratory technique was reduced to a minimum. The usual macroscopic, or external differences in stems

and roots in consequence of liberal and deficient calcium supply were noted before those of microscopic nature were studied. Miss DAY (6) emphasized the former for *Pisum sativum* in recent work, but she reported no significant differences in anatomical structure in these plant parts.

Both microchemical and staining methods for demonstrating calcium and pectate in micro-sections were employed in an attempt to discover any differences in the cell walls. Material from 10-day old



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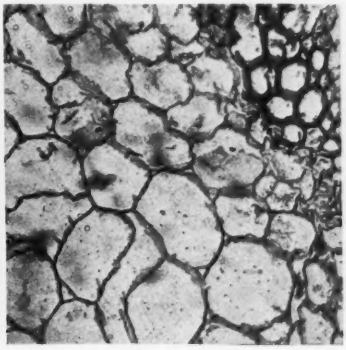

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FIGS. 3, 4.—Cross-section of stems of calcium-starved and calcium-bearing soy bean seedlings (10 days old): fig. 3, calcium-bearing, fig. 4, calcium-starved; ×750.

seedlings is so minute in structure, however, that as yet it has been impossible to record micro-photographically any differences noted. Further work on this phase is being done, and it is expected that this observed histological difference between calcium-starved and calcium-fed soy bean seedlings can be intensified and substantiated by using seedlings more nearly deficient in calcium through growth on sand leached free of calcium with acid and distilled water, and by using seedlings of greater age to intensify their differences.

Summary

1. An experimental study was made of the effect of calcium on nodulation of soy beans on certain acid soils, with the hope of contributing to the knowledge of the rôle calcium plays in inoculation.

2. The divided root system of soy beans, grown in acid soil on one side and calcium-treated soil on the other, gave differences in nodule production to as great an extent as those produced when plants were grown wholly within these same soils. This indicates (1) that calcium plays some physiological rôle in favoring nodulation; and (2) that its effects are local or restricted in increasing the number of root infections, at least within the periods of time used in this experiment.

3. The addition of lime carbonate to an acid soil of pH 5.4, and already infected with legume organisms, gave a very marked increase in nodule production. It suggests that the effect of liming is not necessarily one of keeping alive the bacteria applied as inoculation, since in this case liming increased the number of nodules by organisms originally present in the soil. Evidently the lime carbonate exerted a physiological effect on the plants, and possibly on the organisms, to bring on the greater nodule production.

4. The addition of small amounts of calcium chloride to an acid soil (pH 5.5) increased the viability of the legume organism, *B. radicicola*, of soy beans, applied to the soil by pure cultures, and stimulated nodulation of the host plant.

5. Calcium taken up by the plant in its early growth influenced nodulation, since there was a difference in the nodulation of 10-day old calcium-starved and calcium-bearing seedlings when replanted to an already well inoculated lime-deficient soil of pH 5.4.

6. This functioning of calcium within or through the plant to produce increased nodulation may have a fundamental histological or physiological basis in the plant, since running parallel with the effect on nodulation by calcium given the seedlings, there is a distinct difference in the plant cell wall structure suggested by differences in ease of obtaining micro-sections of the 10-day old calcium-starved and calcium-bearing soy bean seedlings.

UNIVERSITY OF MISSOURI
COLUMBIA, MO.

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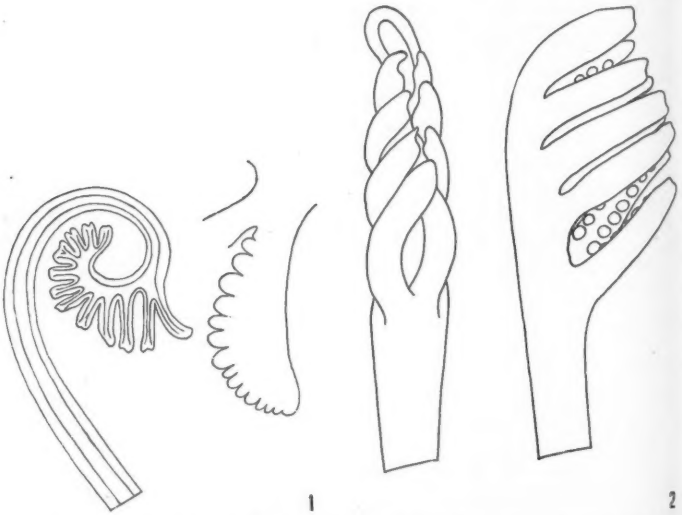
DEVELOPMENT OF SPORANGIUM IN SCHIZAEA RUPESTRIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 398

DORR RAYMOND BARTOO

(WITH TWENTY FIGURES)

The large solitary sporangia of *Schizaea rupestris* are borne in two parallel rows upon the abaxial side of the fertile pinnae. They arise in acropetal succession from marginal cells near the apex, and have already begun their development before the fertile leaves begin

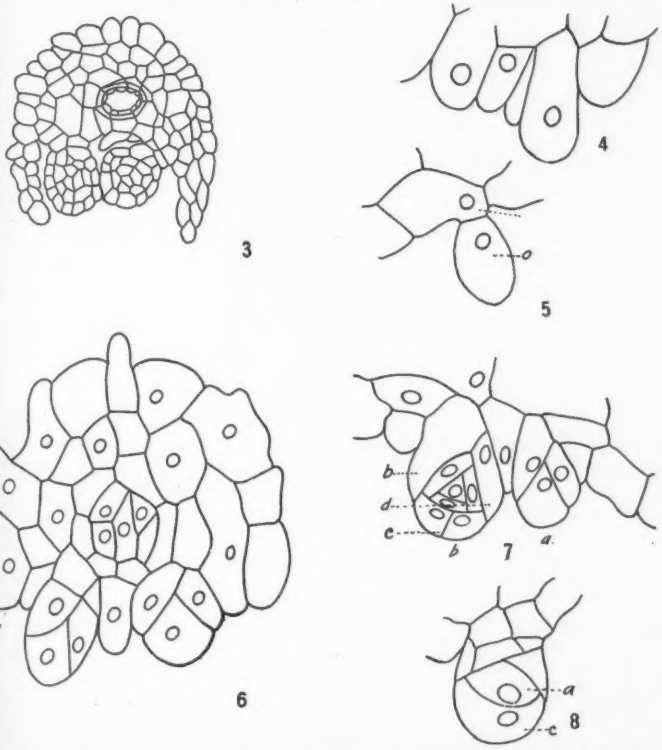


FIGS. 1, 2.—Fig. 1, early development of sporangiophore; fig. 2, later development of sporangiophore showing position of sporangia.

to unfold (fig. 1). During their early stages they become partially inclosed in two ridgelike flaps, the indusia, which are superficial outgrowths from the sporangiophore (fig. 3). Approximately twenty sporangia occur on each pinna, while the number of pinnae per leaf is from ten to twenty (fig. 2).

Initial and indusium

The initial cell of the sporangium appears as a marginal cell near the apex of the pinna, and protrudes until it is hemispherical (fig. 4). Usually a second initial may be recognized in the same transverse



Figs. 3–8.—Fig. 3, transverse section of sporophyll showing sporangia and indusial flaps; fig. 4, sporangium initial; fig. 5, outer (*o*) and inner cell (*i*), developed from initial cell in fig. 4; fig. 6, transverse section of sporophyll showing early development of sporangium and indusial flaps; fig. 7, later development of sporangium; fig. 8, oblique section through young sporangium.

section, thus giving two initial cells of sporangia, each of which represents the latest addition to a sporangial row (fig. 6). When first recognizable these initial cells are very close together, separated only by a single cell, as seen in either transverse or longitudinal sections.

PRANTL[1] (6) shows that the origin of the sporangia is marginal, and that they very soon turn inward toward the lower surface. The present findings agree very closely with BOWER'S (2) illustrations and description of the origin of the sporangium initial and the subsequent development of the indusial flaps. He states:

> If sections of the pinnae be cut when very young the characteristic segmentation of the sporangia is seen to be from marginal cells, and very soon strong growth and division spring up in the adjoining cells, forming a false margin of indusium by which the sporangia are forced toward the midrib and assume a falsely superficial position. One row of them appears on each side of the midrib.

RISE OF ARCHESPORIUM

The first wall laid down in the protruding sporangial initial is level with the epidermal surface, and forms two equal cells, an outer and an inner (fig. 5 *o*, *i*). Just as in the case of other leptosporangiate ferns, the subsequent derivatives of the outer cell become the sporangium, while the inner cell takes no further part in sporangium development.

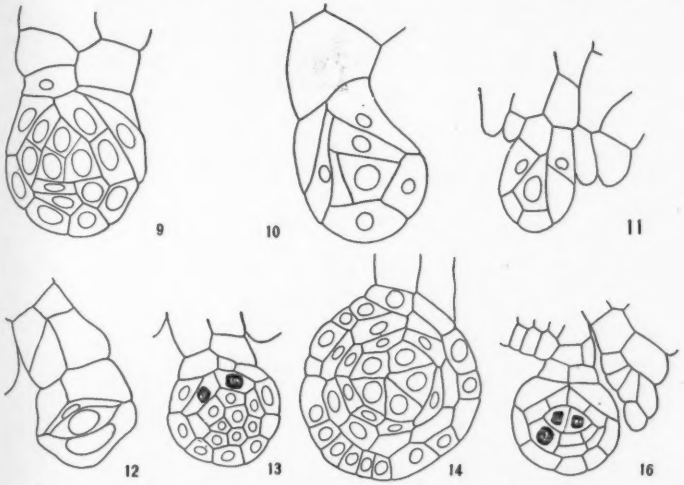
The first wall to appear in the outer cell is oblique, and meets the original transverse wall of the sporangium initial (fig. 7 *a*). A second oblique wall comes in on the opposite side and meets the first, forming a three-sided apical cell (fig. 7 *a*). A third wall then appears parallel to the first. This is followed by a periclinal wall, which gives rise to the cap cell and the archesporium (fig. 8 *c*, *a*). The cap cell very soon becomes arched over the archesporium, and by subsequent divisions gives rise to the greater part of the sporangium wall (fig. 9). The archesporium now functions as an apical cell of the dolabrate type, and from it the tapetal and sporogenous tissues arise.

APICAL CELL

The sporangium arises from a dolabrate apical cell with three cutting faces, rather than from the pyramidal apical cell of the usual fern type. Its relative dimensions and orientation are best shown in figs. 10 and 11, representing longitudinal sections cut respectively parallel and transversely to the axis of the pinna. A transverse sec-

[1] By means of hand preparations, PRANTL showed that the origin of the sporangium is from the marginal cells of the pinnae of all four genera of the Schizaeaceae. His conclusion has been confirmed by BINFORD (1) in *Lygodium*, STEVENS (8) in *Aneimia*, and BOWER (3) in *Schizaea* and *Mohria*.

tion of the young sporangium itself gives the lens-shaped view (fig. 12). BINFORD (1) finds the same type of apical cell in *Lygodium*, in which the development of the sporangium is similar to that observed in the present species of *Schizaea*. On the other hand, BOWER (2) states that the development of the sporangium in the Schizaeaceae follows the usual fern type in its main features, but he describes



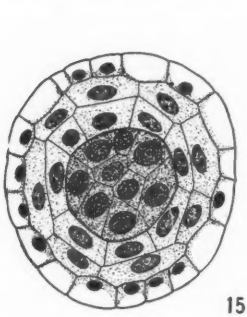
FIGS. 9–14, 16.—Fig. 9, sporangium showing tapetal layer and sporogenous mass of three cells; fig. 10, longitudinal section of sporangium cut parallel to axis of pinna; fig. 11, same as fig. 10, cut transversely to axis of pinna; fig. 12, oblique section of sporangium giving two-sided view of dolabrate apical cell; fig. 13, tapetal cells of sporangium dividing to give two-layered many-celled tapetum; fig. 14, later stage of sporangium as shown in fig. 13; fig. 16, first division of primary sporogenous cell and first division in tapetal layer.

the sequence of wall formation only to the archesporium. Whether the pyramidal or the dolabrate type of apical cell prevails in the Schizaeaceae can only be determined by examination of a great number of species.

SPORANGIUM WALL

The last three cells formed from the outer cell (fig. 7 *b*, *c*, *d*) do not divide periclinally, but by subsequent anticlinal divisions give rise to a many-celled single-layered sporangium wall. As shown by fig. 13, the majority of the cells are derived from the cap cell (fig. 7 *c*).

The contents of the sporangium wall cells are only slightly granular, becoming less so as the sporangium develops, while the cells derived from the archesporium remain highly granular (fig. 14).



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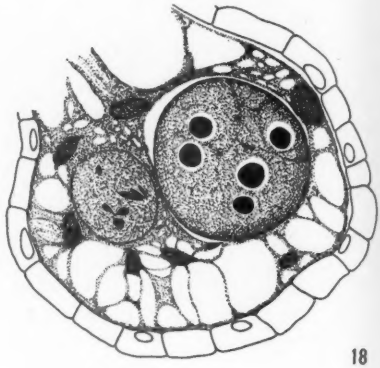

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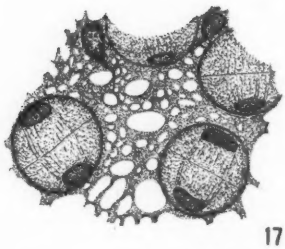

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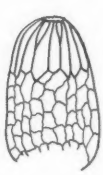

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FIGS. 15, 17–20.—Fig. 15, still later stage showing more dense granular contents of sporogenous mass; fig. 17, spore mother cells forming tetrads; fig. 18, multinucleate condition of some of spore mother cells; fig. 19, sporangium showing terminal annulus; fig. 20, same as shown in fig. 19 after dehiscence.

TAPETUM

Just as the last three cells cut off initiate the sporangium wall, so do the next three cells cut off from the apical cell, in the same serial order, constitute the initial cells of the tapetum (fig. 7). Each divides anticlinally to form a sextet of tapetal cells, and then by periclinal divisions these six cells give rise to two layers of six cells each (figs. 13, 14). Subsequent anticlinal divisions in two planes result in a many-celled tapetum of two layers (fig. 15).

Sporogenous tissue

Upon formation of the initial cells of the tapetum, the apical cell ceases to cut off segments and becomes the primary sporogenous cell. The first division is parallel to the long axis of the sporangium, resulting in two equal cells (fig. 16). Three successive divisions follow, giving rise to sixteen spore mother cells. Occasionally eighteen to twenty spore mother cells were observed. It was noted that the first divisions of the sporogenous tissue were simultaneous with the first divisions of the tapetal initials (fig. 16).

Although at the time of spore mother cell formation all the cells within the sporangium wall have dense granular contents and large nuclei, the cells of the sporogenous tissue are differentiated from the surrounding tapetal cells by their deeper stain (fig. 15); in fact, at no time is it difficult to distinguish the denser sporogenous mass.

As was observed by Binford, subsequent to the formation of spore mother cells there is an enormous increase in the sporogenous mass. The dissolution of the middle lamellae and the consequent rounding off of the spore mother cells go on simultaneously with the breaking down of the massive tapetum. The formation of tetrads and spores quickly follows (fig. 17). The nuclei and cytoplasm of the tapetum flow into the sporogenous mass so that the young spores are surrounded by an abundance of food. Stevens (**8**) has shown that the movement of cytoplasm actually takes place, the tapetal plasmodium creeping in between the spores and holding each one separately imbedded. According to Binford there is a diversion of food material from tapetal to spore formation. First there is a comparatively small amount of growth in the sporogenous mass, with a large growth of the sporangium wall and tapetum (fig. 15), but after the spore mother cells have rounded off and become imbedded in the tapetal plasmodium, growth is rapid and the spore mass becomes comparatively large. Thus the nutrition which is first stored in the rapidly growing tapetal layers is later transferred to the rapidly growing sporogenous mass.

Multinucleate sporogenous cells

It has already been stated that sixteen is the usual number of spore mother cells. Not infrequently, however, wall formation in the

sporogenous mass fails to follow nuclear division. Thus sporangia were occasionally found which contained from two to fourteen spore mother cells, some or all of which were multinucleate. No cases were observed where there was a failure of wall formation immediately following nuclear division of the archesporium. After the second nuclear division one of the walls sometimes fails, however, resulting in a sporogenous mass of three cells, one of which is binucleate. The ultimate result of succeeding nuclear divisions is a sporogenous mass of nine mother cells, one of which contains eight nuclei and is equal in mass to the sum of the other eight uninucleate spore mother cells. It would seem that successive nuclear divisions take place too rapidly for walls to form. Or after the failure of wall formation has once occurred it is probable that the protoplasmic mass is too great for any subsequent nuclear division to form a wall. However, four successive nuclear divisions occur in the sporogenous mass just as in the usual condition of wall formation. Other sporangia were observed in which wall formation had failed to occur after the second successive nuclear division, resulting in one or more four-nucleate spore mother cells. Similarly there were observed cases where wall formation had failed in the third successive nuclear division, giving one or more binucleate spore mother cells. No cases were observed in which the sporangia did not contain some uninucleate spore mother cells; thus wall formation had taken place in at least part of the sporogenous mass (fig. 18).

Stalk

The short stalk, consisting of two or three cells, is derived from the first cells cut off from the sporangium initial (fig. 11). It often remains a single cell in length but sometimes one or more of the stalk cells undergo cell division (fig. 13).

Annulus

The development of the annulus which arises from the cap cell (fig. 8 *c*) has been completely worked out by Prantl (**6**). By divisions of the cap cell fourteen or more elongated ring cells develop (figs. 19, 20). A single three- or five-angled "plate" cell, having one of its angles directed toward the stomium, is formed at the apex of the sporangium (fig. 20).

Relationships

While the nature of the present investigation does not warrant a discussion of the primitive and advanced characters of the Schizaeaceae as a whole, it may be worth while to enumerate some of the outstanding characters of *Schizaea* itself. It is primitive in its simple leaves, its apparent dichotomy, and its open venation. On the other hand, its definite annulus and reduced output of spores (prevailingly 128 per sporangium)[2] relate it to the more advanced leptosporangiate ferns. The greatest reduction of spore output apparently takes place in the presently investigated species (64 per sporangium).

The relationship of the Schizaeaceae to the Marsileaceae, as supported by Campbell (5), is an interesting one. He observed the remarkable similarity between the structure of the very young sporocarp of *Marsilea* and the fertile leaf segment of *Schizaea*, and also noted that the "filiform leaf of *Pilularia* finds its exact counterpart in the sterile leaves of *S. pusilla*."

Bower (2) has made a systematic comparison of the Marsileaceae and the Schizaeaceae, and by way of summary states:

> The result of this comparative analysis is to render support to Campbell's recognition of a real relationship of Marsileaceae to Schizaeaceae, its similarity extending along the whole line of comparison. . . . . In any case the relationship to the Schizaeaceae seems beyond doubt. The Marsileaceae may be held to be a family of the same fundamental type, though specialized for aquatic life, and heterosporous. But it does not seem possible to link them definitely with any single genus of the Schizaeaceae; the similarities are, however, closest to *Schizaea* and *Anemiorrhiza*. In support of the comparison it may be noted that *S. rupestris* is to be found growing in a very moist condition, under dripping rocks, in the Blue Mountains, New South Wales, as a veritable hydropterid in habit.

The present investigation renders even greater support to the proposed relationship between the two families, and, as already suggested by Bower (2), the similarities of Marsileaceae to the genus *Schizaea* are greatest. The species of *Schizaea* which lies nearest to *Pilularia* is probably *S. rupestris*. In the first place, *S. rupestris* has made the greatest advance of all observed species in the reduction of the number of spores per sporangium; 64 as compared with the pre-

[2] Bower (2) found 128 typical for species of *Lygodium*, *Aneimia*, and *Mohria;* Binford (1) found 242–256 by actual count in *L. circinatum;* Tansley (9) counted 90–115 spores per sporangium in *S. malaccana*.

vailing number of 128. This reduced number of spores has reached the upper limit of *Pilularia* (64–32 per microsporangium). Secondly, the multinucleate condition of the spore mother cells of *S. rupestris* may be interpreted as a step toward heterospory (fig. 18). After nuclear division, walls have failed to form and in some cases there is evidence of the disintegration of nuclei within the multinucleate cells. Occasionally four small elongated nuclei are so placed with respect to one another that it would indicate a feeble attempt at tetrad formation (fig. 18).

WILLIAMSON and SCOTT (10) conclude from their investigation of *Calamostachy casheana* that heterospory was reached by the abortion of spores in certain sporangia, the remaining, because of excessive nutrition, becoming "specialized spores." SHATTUCK (7), by controlling cultures of *Marsilia*, obtained all degrees of abortion in the microsporangium. When all the microspores except one aborted it was "about sixteen times as large as a normal microspore," and had acquired other "certain megaspore characters."

The spore output per sporangium in *S. rupestris* has reached the upper limit of that of the microsporangium of *Pilularia*. In the megasporangium of *Pilularia* only one of the spore mother cells develops to the tetrad stage, and only one of the four daughter cells becomes a mature megaspore. It seems probable that the failure of wall formation followed by the abortion of nuclei within the sporangium of *S. rupestris* has become a constant feature in the megasporangium of *Pilularia*. There is little doubt that future investigations upon the origins and development of the tissues of the plant bodies will establish a real relationship between the homosporous Schizaeaceae and the heterosporous Marsileaceae.

Summary

1. The origin of the large solitary sporangium is marginal.

2. The sporangium wall and tapetum arise from segments cut from a dolabrate apical cell.

3. The primary sporogenous cell by three successive divisions gives rise to sixteen spore mother cells.

4. Subsequent to the formation of the spore mother cells and the tapetal plasmodium there is rapid increase in the sporogenous mass.

5. A striking feature is the formation of multinucleate spore mother cells, which is not uncommon.

6. Two features render additional support to the relationship of Schizaeaceae to Marsileaceae proposed by CAMPBELL and supported by BOWER: (*a*) The spore output of *S. rupestris* has advanced to 64 per sporangium as compared with the prevailing number of 128 in most species. This reduction in spore output has reached the upper limit of *Pilularia* (64–32). (*b*) Multinucleate spore mother cells resulting from a failure of wall formation following nuclear division is a step toward the heterosporous condition of the Marsileaceae.

Acknowledgments are due Professor W. J. G. LAND, under whose direction this investigation has been conducted. Helpful suggestions have also been given by Professor C. J. CHAMBERLAIN. Thanks are due to Mr. PATRICK BROUGH of the University of Sydney, who collected and fixed the material used.

TENNESSEE POLYTECHNIC INSTITUTE
COOKEVILLE, TENN.

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FIELD OBSERVATIONS ON PERUVIAN HEPATICAE

GEO. S. BRYAN

(WITH SIX FIGURES)

In 1923 I went as a member of the Botanical Expedition of the Field Museum of Natural History, Chicago, to the Peruvian Andes, to study and collect the Hepaticae of that region. My whole attention was to be given to the cryptogams, while Dr. J. FRANCIS MACBRIDE was to devote himself to the phanerogams. This paper is intended as a preliminary report on the Hepaticae of the regions visited.

Field methods

In order that the specimens of the Hepaticae might be used for morphological study, it was decided to employ the standard combination of chromic and acetic acids (in the proportions 1 gm. chromic acid, 1 cc. glacial acetic acid, 400 cc. water) as a killing and preserving agent. The following equipment, therefore, was carried on the expedition: small phials each containing 10 gm. of chromic acid crystals, two 100 cc. bottles of glacial acetic acid, a 100 cc. glass graduate, and a 1000 cc. bottle. All were carefully packed in special containers. The killing fluid was made up in camp as there was need for it, rain water being collected and employed wherever possible. Several hundred 125 cc. wide-mouthed bottles were used as containers for the specimens. These bottles were packed in light but strong boxes, about eighty bottles in a box, each bottle being carefully padded. The corks were waxed and tied securely down by twine.

Our experience would seem to indicate that glass bottles can be used on expeditions as containers for specimens provided proper care is taken in packing them. The collected specimens remained in the killing fluid for nearly a year without any apparent damage or deterioration. Samples of most of the Hepaticae were sent to Professor ALEXANDER W. EVANS, Yale University, who has kindly provided a preliminary check list of the genera, and in some cases of the species. I take this opportunity of acknowledging my indebtedness to Professor EVANS for this courtesy.

Field stations

CHOSICA.—The expedition landed at Callao on March 6, and soon began work in the vicinity of Chosica, which lies in the foothills of the Andes about 25 miles from Lima. The elevation of Chosica is 2800 feet. The rainfall in this region is generally scanty at all times of the year, consequently the high hills are barren except for cacti, and on some slopes bromeliads. In sheltered pockets and ravines a few shrubs, grasses, and other flowering plants eke out a precarious existence. It was not surprising that field studies failed to locate any liverworts in this region.

MATUCANA.—From Chosica our base of operations was moved into the mountains, to the village of Matucana, with elevation of about 8000 feet. This is an area of almost daily rainfall at this time of the year (March). The contrast with the barren hills about Chosica was marked. Here the steep rugged slopes of the mountains were green with grasses, shrubs, and small flowering plants. Heliotrope was abundant and in full bloom. On exposed and rocky areas cacti and bromeliads were conspicuous. Lichens were everywhere abundant.

Careful search revealed only two liverworts in this region. In various localities, but chiefly in those shaded by rocks or shrubs, grew small mats of *Plagiochasma rupestre* (Forst.) Steph. The production of sex organs had practically ceased, and large numbers of sporophytes, many of them mature, were in evidence. In similar habitats and sometimes associated with *Plagiochasma* was found a species of *Targionia*. It had also passed the stage of sex organ production, and maturing sporophytes were numerous.

RIO BLANCO.—Rio Blanco is 20 miles farther into the mountains, and has an elevation of 11,300 feet (fig. 1). Among the conspicuous flowering plants of this region were long grasses, nasturtiums that clambered over rocks and shrubs, an oxalis with a thick woody stem, a large purple geranium, and, on low areas near the brooks, yellow masses of *Senecio*. The liverworts were also more plentiful here. In damp shaded places grew fine mats of *Plagiochasma rupestre*, and *Targionia* was almost as abundant. At this higher altitude many of the plants were still producing sex organs, and only a few mature sporophytes were visible.

On a sheltered dripping bank, at an altitude of about 12,000 feet, a *Marchantia* was found which has been identified by Professor EVANS as *M. plicata* Nees & Mont. (fig. 2). The plants were smooth in appearance, olive green in color, and were large and vigorous, some of the branches measuring nearly 2 inches across. Many of the plants bore young female receptacles which, like the thalli, were also large. No male receptacles could be located, but that male plants were undoubtedly present was indicated by three greatly enlarged female receptacles crowded with almost mature sporophytes.

FIG. 1.—Difficult terrain at Rio Blanco

In the shade of shrubs and bushes, and on bare spots among tall grasses, at an elevation of more than 12,000 feet, were discovered scattered plants of a very delicate *Fossombronia, F. crassifolia* Spruce. Many of the plants bore mature sporophytes, the round black capsules on relatively long slender stalks being quite conspicuous.

While studying the plants of *Fossombronia* under a large reading glass, I came by chance on a very small thalloid liverwort bearing several sporophytes on a short-stalked receptacle. A considerable area in the vicinity of the discovery and other likely habitats were carefully searched with the reading glass, but only about half a dozen specimens were secured. This small liverwort has proved to be *Sauteria berteroana* Mont., which hitherto has been known only from

Chili. It is possible that this plant has a somewhat wide distribution along the high Andes, since its small size might readily cause it to be overlooked by collectors.

CERRO DE PASCO.—This location was reached by crossing the backbone of the great western range of mountains. The rolling hills about Cerro, which have an elevation of about 15,000 feet, are for the most part clothed with short grasses. Low patches of a woolly cactus form "mountain sheep," and give a curious appearance to some of the slopes (fig. 3). In a cuplike depression among the hills more

FIG. 2.—*Marchantia plicata* Nees & Mont. at Rio Blanco

plants of *Fossombronia crassifolia* were located, but the most interesting event was the discovery of small patches of a *Riccia* on the wet soil in the bottom of the depression. A field examination with a traveling microscope showed that the plants were clearly dioecious. Professor EVANS is of the opinion that this *Riccia* is probably an undescribed species close to the North American *R. austini*. It requires further study.

HUANUCO.—This town, 65 miles northeast of the previous location, lies in a valley at the head of the Huallaga River, one of the tributaries of the Amazon. The elevation is about 7000 feet. The rainfall in this particular region is relatively light, and no liverworts were found except some patches of sterile *Lunularia* on a sheltered moist bank.

MITO.—This village lies 18 miles northwest of Huanuco, at an elevation of about 8500 feet. To the north east the mountain range makes a great horseshoe bend which is furrowed by three canyons. The rainfall is much greater in this bend than in any of the adjacent regions, and the low montaña[1] which clothes the three canyons proved to be an excellent region for collecting. This area yielded more than forty species of ferns, many lichens and mosses, and a variety of liverworts.

On damp shaded soil in the bottom of the most westerly canyon were small patches of *Asterella macropoda* (Spruce)Evans. Many of

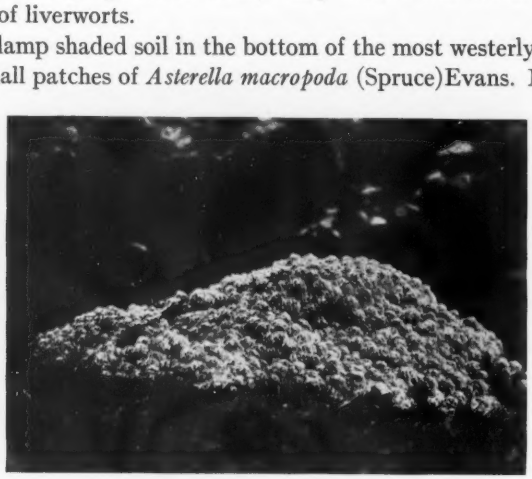

FIG. 3.—Woolly cactus at Cerro de Pasco

these plants bore mature sporophytes, the maximum number on a receptacle being four. Later in other localities excellent specimens were found which showed that the species is monoecious, and that antheridia are borne on slender lateral branches which extend on the under side of the thallus from the midrib to the margin.

Farther up this canyon, on a wet shaded bank of the stream was a mat of *Noteroclada confluens* Tayl., a few of the plants bearing mature sporophytes; and in the immediate vicinity of *Noteroclada* were plants of *Symphyogyna braziliensis* Nees, some of which bore sex organs and others young sporophytes. Another *Symphyogyna*, *S. sinuata* (Sw.) Nees & Mont., was found growing nearby in con-

[1] Mountain forest of the Andes.

siderable quantity in short grass on a wet slope of the canyon. This variable species was here characterized by a stout midrib and delicate but deeply cut lobes which, in some of the specimens, were so pronounced that they might equally well be called leaves.

On many of the shaded rocks in the upper reaches of the canyon, in the precipitous stream bed, and on dripping faces of the canyon wall, were found great masses of *Dumortiera hirsuta* (Sw.) Nees. The plants showed all stages of development of antheridial and archegonial receptacles, and even a few mature sporophytes. *Dumortiera* in some localities is practically an aquatic liverwort; and in another locality it was found thriving on the face of a low cliff, although submerged by a sheet of water that seeped over it. It was also found near a densely shaded waterfall, where it grew on the rocks beside the fall, kept constantly wet by the spray.

In a wet but rather open area beside the stream bed was found a straggling mat of *Megaceros*, and nearby on a deeply shaded clay bank at the foot of a great dripping rock were small patches of *Clevea andina* Spruce. The thallus of *Clevea* here collected is thin, delicate, and pale green in color. A great many of the plants bore mature sporophytes.

Near the more open, basal end of the canyon a small marshy area was practically covered by *Marchantia plicata*. The plants were not so large as those at Rio Blanco, but bore receptacles in all stages of development. On moist but rather open ground was found another species of *Marchantia* which I shall refer to as no. 338, its collection number. Professor EVANS has examined the plant and thinks that it is probably a new species resembling somewhat the West Indian *M. breviloba*. Abundant material was collected in various other localities to be described later, and the morphology of the plant is now being studied. Two other liverworts, an *Anthoceros* and a *Lunularia*, only male plants of the latter being found, complete the list of Hepaticae collected from the soil and rocks about Mito.

The low montaña of the canyons yielded several epiphytic liverworts, the most abundant being species (at least three) of *Metzgeria* which festooned the branches of the trees and bushes. These species of *Metzgeria* were in ideal condition for collection, since they bore sex organs as well as practically mature sporophytes. *Brachiole-*

jeunia laxifolia (Tayl.) Schiffn., a *Plagiochila*, and male plants of a large *Porella* were also somewhat common.

TRAIL TO POZUZO.—Early in May we began the trail to Pozuzo, a small village about 100 miles away across the easternmost range of the mountains and near the navigable waters of the Rio Pachitea. We planned to camp along the trail, stopping and collecting wherever it seemed worth while.

The first few days of travel had little of interest in the way of liverworts, as the mountain slopes were almost as dry as those about Huanuco. In the vicinity of the village of Panao, however, on the side walls of a deep and narrow gully, were found large sheets of *Asterella venosa* (Lehm & Lindenb.) Evans, with receptacles in various stages of development; and in a canyon in the same locality an abundance of *Symphyogyna braziliensis*. *Marchantia* no. 338, bearing mature sporophytes, was collected in considerable quantities beside the trail near Panao, and again below the pueblo of Chaglla which lies about 9 miles beyond Panao.

About Piedre la Grande was found a mixture of grassland and low brush in which a species of *Botrichium* grew in great abundance. In this region only a few liverworts could be found. From a small area of montaña in a ravine came two species of an epiphytic *Plagiochila*, and a *Porella*, all of which bore sporophytes. On the clay banks of a little brook above the trail were scattered plants of *Symphyogyna braziliensis*, which had also been collected at Mito and Panao; and on the steep and deeply shaded muddy banks of the same brook close to the trail were found scattered patches of *Plagiochasma intermedium* Lindenb. & Gottsche. Professor EVANS states that this is the first time the species has been reported from South America. Some of the specimen collected bore sex organs and others mature sporophytes. Although a careful search was made in a number of likely habitats in the vicinity no other plants could be found.

MUÑA.—In the dry scrub leading up to the montaña a great wealth of lichens and probably two species of *Porella* festooned the bushes and low spreading trees. In the cool moist montaña of the canyons were considerable quantities of *Frullania campanensis* Spruce, but *Frullania hians* (Lehm & Lindenb.) Nees & Mont. was

found but rarely and only in small mats. Both species bore practically mature sporophytes. A species of *Metzgeria* as yet undetermined, and *Dicranolejeunea axillaris* (Nees & Mont.) Schiffn. were abundant on the trees in one of the canyons. Both plants had numerous mature sporophytes.

In a canyon about a mile south of Muña *Dumortiera hirsuta* was again found in its usual moist habitat. Associated with *Dumortiera* or in similar habitats were fine mats of *Monoclea gottschei* Lindb. The plants bore mature sex organs and sporophytes in various stages of development. To the east and at an elevation of about 1000 feet above Muña the scrub passes into a magnificent tropical forest with a fine display of tree ferns and a wealth of epiphytes. In this montaña were found, on decaying sticks and leaves, patches of *Megaceros*, and in some rather open places areas of *Anthoceros*. On decaying logs and on mats of dead leaves *Riccardia pinguis* (L.) S.F. Gray was common. Sex organs and sporophytes were abundant on these plants. The list of Hepaticae from Muña is concluded with two species of *Symphyogyna*. A small patch of *S. leptothelia* Tayl. was located on a shaded clay bank in the montaña; and not far away on a wet, grassy clay bank were abundant plants of *S. sinuata*. Both species bore mature sporophytes. The plants of *S. sinuata* were characterized by wavy margins, but no deeply lobed specimens could be found.

Tambo de Vaca.—On June 7 camp was moved 9 miles farther to the east and close to the summit of the great eastern range of the Andes. This tropical forest, beginning at an elevation of about 1000 feet above Muña, extends far on up the mountain, gradually passing near the top into a montaña of low, widely branching trees (fig. 4). Near the crest the montaña is interrupted here and there by small areas of grassland and meadow, and is known by the appropriate name of Tambo de Vaca. The elevation is nearly 13,000 feet. The region about Tambo de Vaca contained a great wealth of epiphytes. The low spreading trees were literally clothed with a variety of ferns, mosses, lichens, and leafy liverworts (fig. 5). The reason for this wonderful display was evident. For much of the year the warm moisture-laden winds from the east drench the top of this eastern

range with dense mists or heavy rains, and even in the dry season the crest is enveloped in swirling clouds and light rains are not infrequent.

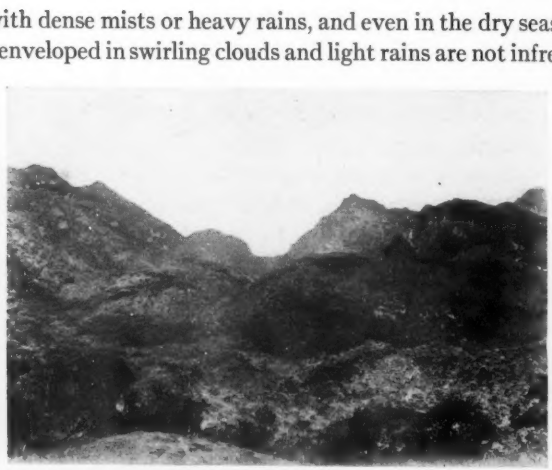

FIG. 4.—Low montaña at Tambo de Vaca

FIG. 5.—At edge of meadow in low montaña; trees covered with ferns, lichens, liverworts, etc.

Unfortunately a box containing, among other things, dried specimens of leafy liverworts from this region was lost in transit to New York, consequently I am able to give but an incomplete list of the Hepaticae from Tambo. One of the most abundant epiphytic liver-

worts was *Frullania brasiliensis* Raddi (fig. 6). In some localities it dominated the epiphytes and in certain localities trees were found entirely covered by it. *Radula ramulina* Tayl. was also quite abundant, but seemed to prefer shaded and less exposed habitats. Among the less common forms were a *Plagiochila*, apparently quite different from the two species found at Piedre la Grande, and a *Trichocolea*. The latter bore mature sporophytes only. *Metzgeria filicina* Tayl. occurred in small amounts in well shaded habitats. Near the trail, at an elevation of about 12,000 feet, on a shaded, wet clay bank and partially hidden in a scanty growth of grass, were some fine speci-

FIG. 6.—Epiphytic vegetation within low montaña

mens of *Symphyogyna brongniartii* Mont. All the plants examined were deeply lobed, and in nearly all cases the lobing extended to the thick stemlike midrib. Superficially these lobes are indistinguishable from leaves, and are exceedingly brittle. The plants bore sporophytes in varying stages of maturity, and it was interesting to note that the sporophytes were in most cases about twice the size of those of *S. sinuata* which was collected in the same vicinity but at a lower altitude. *S. sinuata* and *S. brongniartii* may not be specifically distinct, but certainly the specimens found in this region have very different characters.

Riccardia pinguis, which was quite abundant in the montaña above Muña, was found to extend its range up the mountain to an

elevation of about 12,000 feet. *Asterella macropoda* and a *Marchantia*, probably *M. chenopoda* L., were found near the crest of the mountain at an elevation of nearly 13,000 feet.

The vegetation of the high eastern slopes, which were next traversed, was quite similar to that of the western. First low trees were encountered, covered with ferns, mosses, lichens, and leafy liverworts. There were also the same familiar small areas of grassland and meadow. But the most striking new feature was the luxuriant growth of bamboo, small areas of which had been observed on the high western slopes. The liverworts were disappointingly the same, only one new form being found on the first day's journey, a species of *Dendroceros* which was somewhat abundant in one particular locality near the trail, but bore neither sex organs nor sporophytes.

In the vicinity of Cushi two liverworts were quite common, one being *Asterella macropoda*, already found in various other localities, the other was *Marchantia chenopoda*. In one or two places along the trail near Cushi I observed the now familiar *Dumortiera hirsuta*, *Monoclea gottschei*, and *Riccardia pinguis*, and took a few samples of each.

Near the settlement of Pozuzo fine specimens of *Monoclea* and *Dumortiera* were found in abundance. In relatively dry but shaded habitats were considerable quantities of a very small, delicate *Marchantia* which Professor EVANS has identified as *M. chenopoda*. The plants were all uniformly small and delicate, being much less than half the size of the more characteristic form found at higher elevations. The structure and development of the sporophyte of this small form are being studied and contrasted with *M. polymorpha* in this laboratory, and interesting differences have been found.

TRAIL TO CHINCHAO.—On a densely shaded bank near Chinchao were numerous plants of *Pallavicinia erythropa* (Gottsche) Steph. At this late season of the year they bore sporophytes in various stages of development. In this vicinity was again observed the small delicate form of *Marchantia chenopoda* which had been found near Pozuzo.

UNIVERSITY OF WISCONSIN
MADISON, WIS.

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CURRENT LITERATURE

BOOK REVIEWS

Plant succession and indicators

A volume has just appeared by CLEMENTS[1] which is a combined and condensed edition of *Plant succession*, issued in 1916, and *Plant indicators*, issued in 1920. The impulse for this condensed edition comes from the fact that the supply of original editions of both works is exhausted. Essentially no new material has been added, and considerable portions of the text have been omitted, notably chapters X and XI of the first mentioned book, dealing with succession in North America and Eurasia; also chapters XII and XIV on paleoecology. Chapter IV of the second book, dealing with climax formations of western North America, is also omitted. These particular omissions are made because it is anticipated that in the near future extensive treatises will be published by the authors dealing with these subjects in enlarged style. In order to save additional space, a number of the plates and figures of the former works are omitted, also the indexes. The bibliography is very much reduced. There is, however, an ample table of contents, so that it is not difficult to find any particular subject desired.—H. C. COWLES.

Physiological basis of drought resistance

The excellent monograph of MAXIMOV[2] on the relations of the plant to water, which has just been published in English translation, deserves a wide reading. Written by an eminent Russian plant physiologist and edited by a well-known English ecologist (whose obituary notice we regret to see in the preface), the book has an authoritative point of view in the synthesized field which it covers.

The first half of the work deals with the physiology of the intake and outgo of water by the plant, and with the factors which control those processes. These chapters discuss these matters concisely and critically, and are up-to-date in their treatment. But while they are valuable because they bring all the material conveniently together, they are not so notable as the chapters of the second half of the book, wherein the nature of the drought resistance is considered. Here is

[1] CLEMENTS, F. E., and CLEMENTS, EDITH S., Plant succession and indicators. pp. 460. *pls. 44. figs. 24.* New York: H. W. Wilson Co. 1928. $8.50.

[2] MAXIMOV, N. A., The plant in relation to water: a study of the physiological basis of drought resistance. Ed. by R. H. YAPP. 8vo. pp. 451. *figs. 46.* London: George Allen and Unwin Ltd. 1929. 21*s.* Macmillan Co. 1929. $6.50.

MAXIMOV's real contribution. He offers convincing evidence that the true criterion of xerophytism is the ability of the plant to recover again after complete wilting, and not the ability to prevent that wilting by reducing the rate of transpiration. "All influences which result in greatly increased loss of water by the plant, or a restricted supply of water to the developing leaves, lead to essentially similar changes of leaf structure." These changes ("xeromorphic") tend to facilitate water supply and also to increase gas exchange, and therefore xeromorphous plants have higher, rather than lower, rates of transpiration and photosynthesis.

Xerophytes do not seem to be able to use water from drier soil than other plants. They simply wilt when they can no longer obtain enough water, and are able to recover again later. Xerophytic characters are (1) anatomically: decrease in size of all cells, thickening of cell walls, strong development of palisade mesophyll, denser network of veins, and increased number of stomata per unit area; and (2) physiologically: increase in intensity of photosynthesis and transpiration, in osmotic pressure, and in capacity to endure wilting. TUMANOV has found some evidence that plants may be "hardened" against drought in ways analogous to those of hardening against cold in use now, so that "drought during early stages may be beneficial"! Another concept of great value is that of a critical period of drought for various kinds of crops. Not the total water available, but the time in the development of the plant at which the supply is deficient, seems often to be the determining factor.

Throughout the whole book one is continually aware of the great care which the author has taken to collate all work bearing on the subject, to present data whenever it would clarify the argument to do so, and to analyze data and arguments critically. Indeed, the reader may sometimes decide that the author is not wholly justified in his conclusions, so fully does he present all the evidence. But his reasoning is clear, with rare exceptions. When he takes issue with LIVINGSTON as to the value of the decreasing water content of the leaf in curtailing transpiration, his zeal for his own explanation makes him less clear than usual as to the relative importance of the various regulatory mechanisms in different situations. Consequently we read that "LIVINGSTON's concept of 'incipient drying' is of very limited importance," whereas "a similar view was put forward by SRESNEVSKI some years previously," and "this purely physical regulation of water loss . . . . . established by SRESNEVSKI is of extreme importance," statements whose context does not alter their apparent meanings as here given. MAXIMOV thinks unconsciously only in terms of cases of dry soils, but even here the immediate cause of transpiration decrease must be a shortage of water in the leaf cells. His theory of curtailment of transpiration by shortage of water in the root cells seems to the reviewer to differ little from LIVINGSTON's in the final analysis. But the instance cited is exceptional, and the book is also singularly free from typographical errors.

The inclusion of the great amount of Russian work in this field, normally quite unavailable to English-speaking readers, is a valuable feature of this book;

but all American, English, German, and French work up to 1925 (when the Russian edition was published) with no notable exceptions has been taken into account, and the more important work even up to 1928 has been included in most phases of the subject. The result is an up-to-date, authoritative presentation of an important phase of plant physiology and ecology, with many practical applications suggested in the field of drought resistance.

An extensive bibliography is appended and an excellent index.—H. S. WOLFE.

Phytopathology

Recent volumes by OWENS[3] and BROOKS[4] indicate that the intellectual climate of phytopathology in the United States and in England has definitely changed. Although phytopathology on the continent always considered its content to include any disease and undesirable anomaly no matter how induced, American and English usage irrationally has virtually restricted the content of phytopathology to diseases and anomalies caused by bacteria, certain myxomycetes, fungi, and a few animals such as nematodes. In other words, phytopathology was essentially applied phytobacteriology and phytomycology.

A different trend was indicated by WHETZEL'S laboratory manual[5] and later by HEALD'S excellent manual.[6] With the publication of the volumes by OWENS and by BROOKS, the English language has two more books in phytopathology which, while they emphasize the diseases caused by bacteria and fungi, at least indicate that non-parasitic diseases and diseases caused by algae, for example, are a part of the content of phytopathology. Neither book goes all the way to include the abnormalities caused by insects. Both books preface their special parts with a discussion of the fundamental principles that form the basis of the science. Even though these discussions are far too brief and incomplete, it is to be hoped that they augur that phytopathologists are beginning to look upon their field as more than a congeries of individual diseases.

OWENS' book, like HEALD'S, was written with the need of his classes in mind. Consequently one misses, even in the special parts, detailed discussion of diseases that occur in the south. The arrangement of the special diseases by OWENS is not as sound, considered from the point of phytopathology as a science, as that by BROOKS. There is a definite pedagogical gain in taking up first the non-parasitic diseases. A brief presentation of the structure and life histories of the classes of parasites would have enhanced the value of OWENS' volume as a textbook. However, the book fills a real need, especially for agricultural students, who after all, are not so much interested in the science per se as they are in the diagnosis and control of plant diseases.

[3] OWENS, C. E., Principles of plant pathology. pp. xii+629. *figs. 222*. New York: John Wiley and Sons. 1928.

[4] BROOKS, F. J., Plant diseases. pp. vii+386. *figs. 62*. Oxford Press. 1928.

[5] BOT. GAZ. **81**:470. 1926.

[6] BOT. GAZ. **82**:443. 1926.

BROOKS' volume is essentially a brief discussion of the diseases which occur in crops in the British Empire. It is surprising that such a volume has not appeared before this. The discussions, in the main, are brief descriptions of the individual diseases. It is gratifying that this volume has come from one of the students of H. MARSHALL WARD.—G. K. K. LINK.

Control of pathogens

An excellent volume has been prepared by TRAPPMAN[7] on control of the pathogens (Pflanzen Schädlinge) of plants, in which he includes all living agents, parasitic and non-parasitic, which injure plants. Considerable attention is devoted to control of those factors which affect the susceptibility and resistance of plants to attack by injurious agents, and the occurrence and abundance of these. In doing this he incidentally considers control of non-parasitic diseases. The introductory chapters are devoted to (1) significance and aim of plant protection; (2) concepts of disease and injury; (3) causes of injuries (in the sense of damage); (4) parasite and host; (5) disease symptoms; (6) occurrence and distribution of pathogens. Control is treated under the main headings: cultural practices; biological methods; technical methods using physical means; technical methods using chemical means; evaluation of these methods; and organization of control.

The influence of MORSTATT'S volume is very marked in the introductory chapters. Account has been taken to a surprising extent of plant disease control methods and research in the United States. The volume is a timely and useful compilation of widely scattered but highly important data for anyone concerned with the control of plant diseases. It is significant that it appeared as the 8th volume in the series *Chemie und Technik der Gegenwart*.—G. K. K. LINK.

Manual of microbiology

The volume by FRED and WAKSMAN[8] is a useful addition to the literature on the laboratory phases of microbiology. It gives adequate and clear directions for the standard laboratory manipulations, such as preparation and formulas for culture media, methods and formulas of staining, qualitative and quantitative methods of analysis, and the study of microorganisms in the soil.—G. K. K. LINK.

NOTES FOR STUDENTS

Protoplasmic ether-soluble constituents.—An interesting and valuable study of the ether-soluble constituents of cabbage leaf cytoplasm has been made by CHIBNALL and CHANNON, who began to publish on this subject[9] about two

[7] TRAPPMAN, W., Schädlingsbekämpfung. 8vo. pp. viii+446. *figs. 68*. Leipzig: S. Hirzel. 1927.

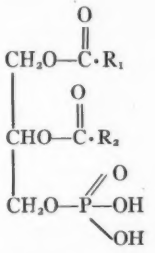
[8] FRED, E. B., and WAKSMAN, S. A., Laboratory manual of general microbiology. New York: McGraw-Hill. pp. viii+145. *figs. 19*. 1929.

[9] CHIBNALL, A. C., and CHANNON, H. J., The ether-soluble substances of cabbage leaf cytoplasm. I. Preparation and general characters. Biochem. Jour. **22**:225–232. 1927.

years ago. After preparation of the leaf cytoplasm from green unheaded cabbage as a solid cytoplasmic mass, it was extracted with anhydrous ether, 149 gm. of cytoplasm yielding about 17.6 gm. of ether-soluble extractives.

In general, the ratio of protein in the cytoplasm to the ether-soluble extract was about 3:1. About 42 per cent of the fatty material contains phosphorus, and 58 per cent is phosphorus-free. Approximately 17 per cent of the extract is unsaponifiable, and fatty acids make up about 23 per cent of the total. The phosphorus-containing fraction[10] was precipitated with acetone. Examination of this acetone-precipitable fraction showed that there were no phospholipins present, but that all the phosphorus was tied up with calcium, glycerol, and fatty acids. The main constituent was found to be the Ca salt of diglyceridephosphoric acid, with some Ca monoglyceride phosphate.

A more detailed examination[11] of the Ca salts of diglyceridephosphoric acid led to the conclusion that the free acids and its salts should be classed, according to THUDICHUM'S early classification, as phosphatides; and, since the acid is known to be the parent acid of lecithin and kephalin, the authors propose to call it phosphatidic acid, and the Ca salt, calcium phosphatidate. The structure of the free acid is:



It differs from the acid of lecithin and kephalin in that these have both saturated fatty acids in equimolecular proportions, while phosphatidic acid is made up much more completely of unsaturated fatty acids, linolic and linolenic mainly, in ester linkage with the glycerol.

The phosphorus-free fraction, the 58 per cent not precipitated by acetone, was examined for fatty acids.[12] The material was saponified, and the fatty acids freed were identified as far as possible. The greater amount of these were found to be unsaturated acids, also linolic and linolenic probably. The saturated acids were mainly palmitic and stearic. The presence of oleic acid was not demonstrated, although it may be present in small amounts, and arachidonic acid is absent.

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[10] CHIBNALL, A. C., and CHANNON, H. J., II. Calcium salts of glyceridephosphoric acids. Biochem. Jour. 21:233–246. 1927.

[11] ———, IV. Further observations on diglyceridephosphoric acid. Biochem. Jour. 21:1112–1120. 1927.

[12] ———, III. The fatty acids. Biochem. Jour. 21:479–483. 1927.

Two non-phosphorus-containing compounds have been isolated from the phosphatide fraction, where they occur because of their similar insolubility in ether-acetone. These have been identified[13] as one of the higher paraffins, n-nonacosane, $C_{29}H_{60}$; and di-n-tetradecyl ketone, $C_{14}H_{29}\cdot CO\cdot C_{14}H_{29}$. These two substances constitute nearly half of the "phosphatide" fraction, and have previously been isolated only in small amounts in plant fats. The determination of the number of carbons in the molecule was done by X-ray analysis. The ketone was separated from the paraffin through the solubility of its ketoxime in a mixture of equal parts of light petroleum and acetone, a solvent in which the paraffin remains practically insoluble. The ketone was then regenerated, and found to have a melting point of 80.5–81° C. X-ray analysis again showed the ketone to contain twenty-nine carbons, and the CO group in the center of the chain. But because of the length of the carbon chain in the ketone, it was difficult to decide whether it was the symmetrical $CH_3\cdot(CH_2)_{13}\cdot CO\cdot(CH_2)_{13}\cdot CH_3$ or the unsymmetrical palmytyl-myristyl ketone, $CH_3\cdot(CH_2)_{14}\cdot CO\cdot(CH_2)_{12}\cdot CH_3$. Both of these compounds were then synthesized, and it was found that the latter has a different melting point (74–74.5° C.) from the naturally occurring ketone, while the symmetrical synthetic ketone had the same melting point as the one isolated from the cabbage.

In the summary and general conclusions[14] they bring out many interesting points in the physiological chemistry of plant fats. They also suggest that some term is needed to denote an ether extract, which is irrespective of the contents, and which does not connote any particular class of substances. They prefer that to BLOOR's term "lipide," which was proposed a few years ago.—C. A. SHULL.

[13] CHIBNALL, A. C., and CHANNON, H. J., V. The isolation of n-nonacosane and di-n-tetradecyl ketone. Biochem. Jour. 23:168–175. 1929.

[14] ———, VI. Summary and general conclusions. Biochem. Jour. 23:176–184. 1929.